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***Phylogeography, Kinship and  
Molecular Ecology of Sperm Whales  
(Physeter macrocephalus)***

by

**Daniel Thomas Engelhaupt**

**Submitted in partial fulfilment of the requirements  
for the degree of Doctor of Philosophy**

at

**University of Durham  
Department of Biological Sciences  
2004**



- 6 DEC 2004

## ***Declaration***

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## ***Abstract***

The molecular ecology for sperm whales (*Physeter macrocephalus*) in the northern Gulf of Mexico was investigated in detail using a suite of molecular markers. In addition, several genetic related aspects for the Mediterranean Sea, North Sea and the North Atlantic Ocean putative sperm whale populations were described. These analyses have provided new insights requiring proper management to ensure the survival of the northern Gulf of Mexico sperm whale stock in an area of increasing industrial activity.

The majority of surface behavioural reactions witnessed after biopsy darting were mild and short-term. No significant differences were determined between males and females and repeat sampling events on the same individual did not lead to an increase in the response level.

Population structuring between the four putative populations, with respect to mtDNA, was highly significant and warrants the classification of each putative population as a unique stock for management purposes. The majority of Gulf of Mexico samples were from females and young males believed to be sexually immature based on rough size estimates. Incidental resampling of a few individuals over periods of days, months and years adds support for site-fidelity to the northern Gulf of Mexico exhibited by at least some whales. Although our sample set compares a more restricted geographic area than previous studies, the lack of significant nuclear differentiation between neighbouring populations suggests that sexually mature males disperse from their natal populations and spread their genes to the more philopatric females.

The genetic composition of Gulf of Mexico sperm whale groups fits the mixed sex and bachelor group type so common in other areas of the world, while the two all-male North Sea stranding groups fit the bachelor group scenario. Relatedness within the Gulf of Mexico female-dominated groups was significantly greater than that found between groups, but still surprisingly low and composed of both single and multiple matriline. Highly related whales (i.e. parent-offspring) were present within groups, but infrequent. The most common relationship found was that of half-siblings. The all-male bachelor groups were comprised of multiple matriline and members were generally unrelated, although cases for half-sibling pairs were present.

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## ***I. General Introduction***

Several species of cetaceans are notoriously difficult to study as they spend the majority of their time underwater and inhabit deepwater offshore areas that require high-cost means to gain even a glimpse into their rather secretive lives. While data gathered by researchers aboard whaling vessels primarily between 1950 and 1980 did provide basic information with regards to reproduction, morphology and movement patterns through the use of discovery tag recaptures, an accurate assessment of social and population structure among free-ranging sperm whales (*Physeter macrocephalus*) proved illusive. Pioneering efforts by Jonathan Gordon and Hal Whitehead that began in the 1980s provided the initial non-lethal steps towards unravelling fine-scale social and population structure details for free-ranging whales. Whitehead's long-term study of sperm whales found off the Galapagos Islands and Ecuador provides one of the most significant contributions towards understanding how free-ranging sperm whales live.

The incorporation of molecular markers is a relatively young technique that has the potential to provide a wealth of information with respect to both social and population structure for 'difficult to study' species. With the advent of the polymerase chain reaction, minute amounts of DNA collected from sources ranging from skin to bone can be replicated to produce a viable sample for analysis. The analysis of nuclear DNA, mtDNA and sex-specific genetic markers provides information on identity (Paetkau and Strobeck 1994), gender (Berube and Palsboll 1996), kinship (Blouin et al. 1996; Richard et al. 1996a; Ralls et al. 2001), mating systems (Girman et al. 1997; Fabiani 2002), reproductive success (Gemmell et al. 2001), philopatry and dispersal (Lyrholm and Gyllensten 1998; Lyrholm et al. 1999; Fabiani et al. 2003).

### **I.1 SPECIES DESCRIPTION**

Sperm whales are the largest member of the Odontocetes (toothed whales) and can generally be found occupying deepwater habitats (between 200 and 1000 meters) around the globe ranging from the equator to the edge of the pack ice (Rice 1989). Sperm whales exhibit the greatest degree of sexual dimorphism among cetaceans, with a physically mature male reaching over three times the mass and nearly 1.5

times the length of a mature female (Best 1979; Rice 1989). They are truly unique in their appearance with an enormous head that comprises approximately one-third of their total body length (Figure I.1). In most areas of the world the primary food of sperm whales appears to be meso and bathypelagic cephalopods (squid) with mantle lengths of 0.2 to 1 metre in length (Clarke 1966; Clarke 1980). However, the consumption of fish has been observed and may be an important part of the sperm whales diet in the North Atlantic, North Pacific and off New Zealand (Kawakami 1980; Rice 1989).

Sperm whales were the focus of two intensive hunting periods. Both 'open boat' and 'modern mechanized' whaling eras focused on sperm whales to provide sought after oil to lubricate machine parts and light lamps, in addition to harvesting ambergris (a wax-like substance that originates in the intestine) which was used as a fixative in perfumes and spermaceti oil for fine candles. After the large baleen whale stocks were depleted in the early 1960s, mechanized whaling focused its efforts on sperm whales and animals were taken at a rate of over 20,000 per year (Best 1983). Whalers described areas where sperm whales seemed most plentiful as "grounds" (Townsend 1935). Sperm whales often formed concentrations within these grounds, which were the primary targets during these relatively recent whaling operations. Concentrations may be associated with steep underwater topography, high productivity and oceanographic fronts such as cold-core (cyclonic) eddies (Jaquet and Whitehead 1996; Biggs et al. 2000). Recent post-whaling estimates by Whitehead (2002) place the current numbers of sperm whales around the globe at approximately 360,000. This is a stark contrast to the previous post-whaling global abundance estimate of nearly 2,000,000 (Evans 1987; Rice 1989; Berta and Sumich 1999).

Sperm whales are among the most social of the great whales, with adult females and sub-adults of both sexes associating in social groups and long-term units, while adult males appear to rove over large distances (in some cases between oceans (Ivashin 1981)) on their own (Best 1979; Rice 1989; Whitehead 1993; Whitehead and Weilgart 2000). Aside from the solitary or occasional pairs of sexually and physically mature males (Best 1979; Rice 1989), sperm whales are primarily found in mixed sex social groups believed to be comprised of constant companions and casual acquaintances and all male (bachelor) groups (Best 1979;



Figure I.1 Sperm whale illustration (courtesy of Pieter Folkens).

Whitehead and Arnborn 1987; Whitehead et al. 1991; Childerhouse et al. 1995; Lyrholm and Gyllenstein 1998; Lettevall et al. 2002). Mixed groups primarily inhabit low-latitude waters, while sub-adult males are believed to disperse from their natal groups and tend to inhabit more polar latitudes as they age and grow (Best 1979; Rice 1989). Large sexually and physically mature males eventually return to lower latitudes to breed with females (Best 1979, Whitehead 1993; Christal and Whitehead 1997; Whitehead and Weilgart 2000).

## I.2 THE USE OF MOLECULAR TECHNIQUES TO STUDY CETACEANS

### I.2.1 Population Structure

The allocation of endangered species into particular ‘stocks’ or populations based solely on geographic boundaries seems illogical for most marine mammals given their huge potential for movement. Genetic analyses provide a more suitable means of assessing biologically significant population subdivisions. Significant subdivisions within and among populations seen via an examination of gene frequencies provides a fundamental tool for the management of exploited and protected species. The differentiation of gene frequencies within and among populations can be a result of gene flow via migration of individuals or their gametes, random genetic drift, natural and sexual selection modes, mutations, and genetic recombination opportunities that have been mediated by the mating system (Avice 1994).

Female philopatry and male dispersal are the expected patterns of dispersion for mammalian species based on theoretical considerations (Greenwood 1980). The differences in dispersal between males and females may influence how populations are structured from a genetic perspective. Population structure affected by gender-based dispersal is particularly visible when one compares the haploid and maternally inherited mtDNA with the bi-parental nuclear genome (Avice 1994). If females are philopatric and males disperse, then one expects to find more variation between putative populations with respect to mtDNA and less variation with respect to nuclear DNA. Previous cetacean studies on humpback whales (*Megaptera novaeangliae*: Palumbi and Baker 1994; Baker et al. 1998), fin whales (*Balaenoptera physalus*: Berube et al. 1998) and sperm whales (Lyrholm et al. 1999) using genetic techniques based on mtDNA and nuclear DNA provide valid support for this sex-



biased dispersal scenario. Lyrholm et al.'s (1999) sperm whale study examined population structure on a very broad global scale by comparing a collective set of samples from the North Pacific, North Atlantic and Southern hemisphere oceanic populations. Whether geographic structuring was present within geographic areas of the North Atlantic was untested and thus deemed a priority for sperm whale management related issues.

### **1.2.2 Group Composition**

Sperm whale mixed groups tend to be found in low latitude warmer waters while young all-male bachelor groups and solitary males tend to frequent higher latitudes (Rice 1989). Upon reaching sexual and physical maturity, males return to lower latitudes to breed with females (Best 1979; Rice 1989; Whitehead 1993). Berzin's (1972) idea of sperm whales forming a 'harem band' mating system, with a male dominating a group of females, has since been replaced with a widely accepted polygynous system where sexually mature males only temporarily associate with groups of females in oestrous (Best 1979; Rice 1989; Whitehead 1993; Christal and Whitehead 1997; Lyrholm et al. 1999; Whitehead and Weilgart 2000). The early studies that identified long-term relationships between females (Ohsumi 1971), the presence of juveniles and calves of both sexes and adult females (Best 1979) and what appeared to be cooperation among individuals within groups (Caldwell and Caldwell 1966) led several researchers towards the premature conclusion that sperm whale groups were strictly matrilineal in structure. While it has been noted that the banding together of females into groups may indeed support cooperative foraging, communal care of calves, and provide a collective defence mechanism to defend against predators (Best 1979; Arnborn et al. 1987; Whitehead et al. 1991; Whitehead 1996); a purely matrilineal group structure where females remain with their mothers has since been rejected (Richard et al. 1996a; Lyrholm and Gyllenstein 1998; Christal 1998). The stable social unit appears to present the most probable case for highly related members, although transfers of unrelated whales between units does exist (Christal 1998). In addition to avoiding inbreeding with one another, close kin in vertebrates tend to cooperate and associate more than unrelated individuals (Emlen 1997). The extent of kinship structure that underlies the observed social behaviour of sperm whale groups is fundamental towards understanding the evolution of social

organization and may have important implications as to how to best manage putative populations (Pamilo 1989; Queller and Goodnight 1989).

### **I.3 OCCURRENCE IN THE NORTHERN GULF OF MEXICO**

The continental slope in the north-central and western Gulf of Mexico is an area that supports a diverse cetacean community (Mullin et al. 1994; Davis and Fargion 1996; Jefferson and Schiro 1997; Waring et al. 2001). Nineteen species of cetaceans occur in this area including endangered sperm whales. Although sperm whales are listed as the most widely distributed and abundant of the ‘great whale’ species in the northern Gulf of Mexico, the current minimum population estimate is only 411 whales (Waring et al. 2001). Sperm whales were hunted commercially in the Gulf of Mexico up until the early 1900s, but the actual number of whales harvested is unknown (Townsend 1935). During the 1992-1998 GulfCet I and GulfCet II programs and surveys conducted by the National Marine Fisheries Service (NMFS), seasonal aerial and vessel surveys found whales primarily concentrated over or near the continental slope, with the majority located over the 1000-m depth contour south of the Mississippi River Delta (Sparks 1997; Davis et al. 1998; Waring et al. 2001). The presence of sperm whales throughout the seasons, resightings of photographically identified individual sperm whales occurring near the Mississippi River Delta and sightings of known individuals across seasons provides speculation that a ‘stock’ of sperm whales may exhibit some degree of philopatry to particular deepwater areas in the northern Gulf of Mexico throughout the year (Shmidley and Shane 1978; Mullin et al. 1991; Sparks 1997; Davis et al. 1998; Weller et al. 2000). Recent studies suggest that an association between bottom topography, out-flow of the Mississippi-Atchafalaya River, eddy formations and the Loop current may be key factors as to why sperm whale congregate in deepwater areas of the northern Gulf (Sparks 1997; Davis et al. 1998; Biggs et al. 2000). The sperm whale’s primary prey consists of mesopelagic and benthypelagic squid (Clark 1980), both of which are found in the northern Gulf of Mexico. An adult sperm whale consumes up to 0.91 metric tons of squid per day (Würsig et al. 2000) and the Mississippi River Delta area may provide a concentration of whales with a significant and reliable year-round food source suitable to meet these enormous needs.

#### **I.4 WHY STUDY SPERM WHALES IN THE GULF OF MEXICO?**

For over 30 years, waters over the continental shelf off the coasts of Texas and Louisiana have been an important area for offshore oil and gas industry activities. As supplies are gradually depleted over the continental shelf, seismic exploration vessels have ventured into deepwater areas in search of alternative reservoirs to meet the public's ever increasing demands. The relatively recent and continuous discoveries of significant oil and gas deposits found in deepwater areas past the continental shelf and the inconceivable leaps in deepwater drilling technology devised to extract those deposits from areas that were once deemed inaccessible may conflict with what is believed to be critical habitat utilized by endangered sperm whales. The northern Gulf of Mexico sperm whale stock appears to be highly aggregated with most encounters occurring over or near the 1000 meter depth contour (Waring et al. 2001). Within the last decade, oil and gas related activities in these previously untouched areas have significantly increased with respect to both the number of platforms and seismic vessels in operation.

Seismic exploration, sonar and shipping have all been shown to impact cetacean species. The effects of noise on marine mammals range from injury to death (e.g. Cuvier's beaked whales in Greece and the Bahamas, Frantzis 1998; Balcomb and Claridge 2001); altered vocal behaviour (e.g. humpback whales, Miller et al. 2000) and general avoidance of noise sources (Malme et al. 1984; Richardson et al. 1995). A number of observations have indicated that sperm whales are highly acoustically sensitive and may be disturbed by unusual sounds. Gordon et al. (1992) and Richter et al. (2002) showed that sperm whales in New Zealand changed their vocal behaviour when whale watching vessels were present. Sperm whales are truly unique in a variety of biological aspects and it's this uniqueness that may make them particularly susceptible to disturbance caused by an acoustic source. They rely on acoustically-oriented vocalizations consisting of repetitive clicks and creaks that are thought to serve as a means of long and short range echolocation during foraging dives averaging 40 minutes and depths of several hundred meters (Gordon et al. 1992; Jaquet et al. 2001). However, sperm whales also produce complex patterns of clicks, called codas, which may serve as a means of communication among these

extremely social animals. It may be that not one, but perhaps a combination of biological and social factors make sperm whales susceptible to anthropogenic disturbances.

As an endangered species, sperm whales are fully protected under both the United States Marine Mammal Protection Act and the Endangered Species Act. Endangered species rely on management efforts based on and shaped by research to ensure their survival. The implementation of a multidisciplinary research program was established in 2000 to investigate possible interactions between sperm whales and offshore oil-related activities. One of the key components was to provide a genetic assessment of how this northern Gulf of Mexico stock of sperm whales differed from nearby geographic populations such as the North Atlantic stock. The conservation of genetic diversity among populations helps to ensure the long-term survival of species by preserving the species' ability to respond to changing environments over time, and this is a primary objective of strategies to preserve biodiversity. A deeper understanding of the population and social structure of sperm whales in the Gulf of Mexico is to be integrated with a variety of research projects (e.g. behavioural ecology, habitat use and distribution and abundance estimates) in order to establish realistic global conservation and management strategies. Such information is vital for creating meaningful management strategies for these animals in general, and relative to petroleum exploration and production in particular. In addition, the project provides an important component of a long-term monitoring program that will be needed to ensure that oil and gas exploration and development in the northern Gulf do not have significant adverse effects on any marine mammal species or population.

## **1.5 THESIS AIMS**

As a result of the lack of required information available for management to base decisions on, the primary objective of this research is to describe the genetic structure for the northern Gulf of Mexico stock of endangered sperm whales with respect to both group composition and population structure. In particular, group and cluster compositions were examined from both a gender and genetic relatedness perspective, while the assessment of mtDNA and nuclear DNA genotypes within and between putative populations were analyzed to provide resolution with respect to

how stocks are structured. Assessment of behavioural reactions to biopsy sampling has been included to provide reassurance that this technique provides a useful and relatively non-invasive means of collecting DNA from free-ranging whales.

This study aimed to describe the following aspects that are detailed in separate chapters:

**1) Genetic structure of four putative geographic sperm whale populations**

Previous results for sperm whale population structuring on a global scale based on both matrilineal and bi-parental genetic markers are consistent with the expectation of greater female than male philopatry in this species (Lyrholm and Gyllenstein 1998; Bond 1999; Lyrholm et al. 1999). A sufficient sample size allowed for an examination of structuring within sub-areas of the North Pacific (Lyrholm et al. 1999), however, genetic structuring within areas of the North Atlantic and Southern hemisphere was not possible due to the lack of sample material.

This chapter quantifies the level of geographic structuring and genetic variation among four putative sperm whale populations located in the Gulf of Mexico, Mediterranean Sea, North Sea and the North Atlantic by examining the maternally inherited mtDNA and multiple polymorphic microsatellite loci from the bi-parental nuclear genome. This comparison provides a genetic perspective towards understanding how male and female patterns of dispersal influence population structure within this species. Finally, this chapter provides an important insight as to what extent sexually mature males may be distributing their genes to multiple geographic populations. If sexually mature roving males spread their genes to multiple geographic populations, then this should have consequences on the level of nuclear DNA variation that is present within and between populations.

**2) Composition of sperm whale groups and clusters in the northern Gulf of Mexico and the North Sea**

Sperm whales exhibit a cosmopolitan distribution with females and young males remaining in more tropical and subtropical waters, while larger males increase their range into more polar latitudes as they age and grow (Best 1979; Leatherwood and Reeves 1983; Rice 1989). Apart from the solitary or occasionally paired sexually

and physically mature males, sperm whales are most often found in mixed sex and all male (bachelor) groups (Best 1979; Whitehead and Arnborn 1987; Rice 1989; Childerhouse et al. 1995). Previous genetic-based findings suggest a significant level of relatedness among female dominated mixed sex social group members (Richard et al. 1996a; Lyrholm et al. 1996, 1999; Christal 1998; Bond 1999). A better comprehension of the relatively unstudied groups found in the northern Gulf of Mexico stock was deemed an important and necessary step towards understanding the extent of group structure for these speculated 'resident' whales.

This chapter utilizes molecular markers to combine gender information with genetic relatedness among individuals sampled from both clusters and groups to provide a more detailed assessment of how a groups or clusters of sperm whales within the northern Gulf of Mexico are composed. Relatedness values were tested for members found both within and between groups and clusters to assess whether genetic patterns influence social structure (pending long-term association analyses). Finally, this chapter tests whether two bachelor groups of all male sperm whales stranded on the North Sea coast exhibit some degree of relatedness between whales within each group.

### **3) Surface reactions to biopsy sampling**

The collection of skin from free-ranging cetaceans requires a technique that minimizes the disturbance to the whales, while obtaining a satisfactory sample for research purposes. Biopsy sampling has been done extensively on a variety of cetacean species (Palsboll et al. 1991; Weinrich et al. 1991; Baker et al. 1994; Weller et al. 1997; Hoelzel et al. 1998b) and previous work shows that the majority of reactions are mild and short-term (Best and Butterworth 1980a; Brown et al. 1991; Weinrich et al. 1991; Weller et al. 1997).

This chapter examines surface behavioural reactions exhibited by sperm whales during biopsy sampling events. Assessments of reactions are provided as a means of reassuring sceptics that biopsy sampling is an important and relatively non-disturbing means of collecting high quality tissue that provides the necessary quantity and quality of DNA to be used for genetic analyses.

## ***II. Materials and Methods***

### **II.1 PRIMARY RESEARCH LOCATIONS**

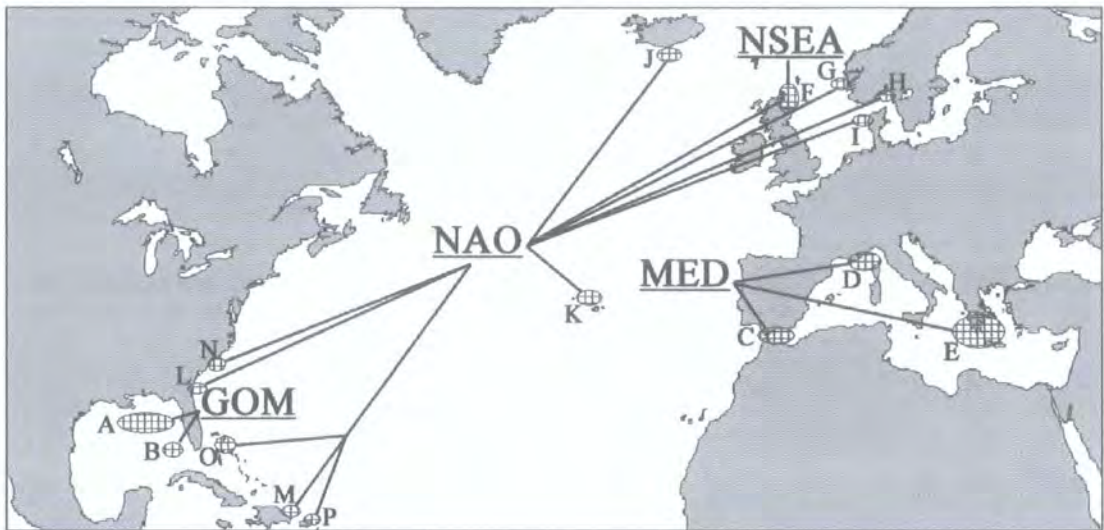
Due to the low minimum population size estimate for sperm whales found in the northern Gulf, emphasis was placed on understanding the population and social structure status of whales reliably seen over the continental slope during National Marine Fisheries Service (NMFS) seasonal cruises (Waring et al. 2001). Although the focus of this research was on whales in the northern Gulf of Mexico (GOM), tissue samples for DNA analysis were collected from both free-ranging and stranded sperm whales distributed throughout the northern GOM, Mediterranean Sea (MED), North Sea (NSEA) and North Atlantic Ocean (NAO) (Figure II.1). Offshore fieldwork in the Gulf was conducted during June – July 2000, March – April 2001, and July – August 2001 aboard the National Oceanic and Atmospheric Administration (NOAA) research vessel *Gordon Gunter*; a 68-m converted U.S. Navy T-AGOS 13 class vessel built for Navy ocean surveillance missions. This ship is ideal for detecting whales either acoustically (using a five element towed array) or visually (using three sets of 25 X 150 ‘Big-Eye’ binoculars and multiple sets of 7 X 50 hand-held binoculars 15 m above the surface of the water on the flying bridge). During June – July 2002 and August – September 2002, the Texas A&M University’s research vessel *Gyre* was utilized as a base for offshore operations using the same sperm whale detection techniques as used on the *Gordon Gunter*. Upon detecting whales, a 7 meter rigid-hull inflatable boat (RHIB) with twin outboard 135 hp Mercury engines was lowered from the primary research vessel for biopsy and sloughed-skin collection and photoidentification (photoID) approaches on an opportunistic basis.

### **II.2 TISSUE COLLECTION AND PRESERVATION METHODS**

#### **II.2.1 Sampling of Free-Ranging Whales**

The majority of tissue samples were collected from free-ranging whales in the six distinct geographic areas (A, B, C, D, E and F) depicted in Figure II.1 (detailed sample locations for tissues collected in the GOM, MED and NSEA are provided in Chapter 4). Sequence results and published data for areas G, H, I, J, K, L, M, N, O and P made up the majority of the NAO dataset. A total of 161 biopsy (n=118)

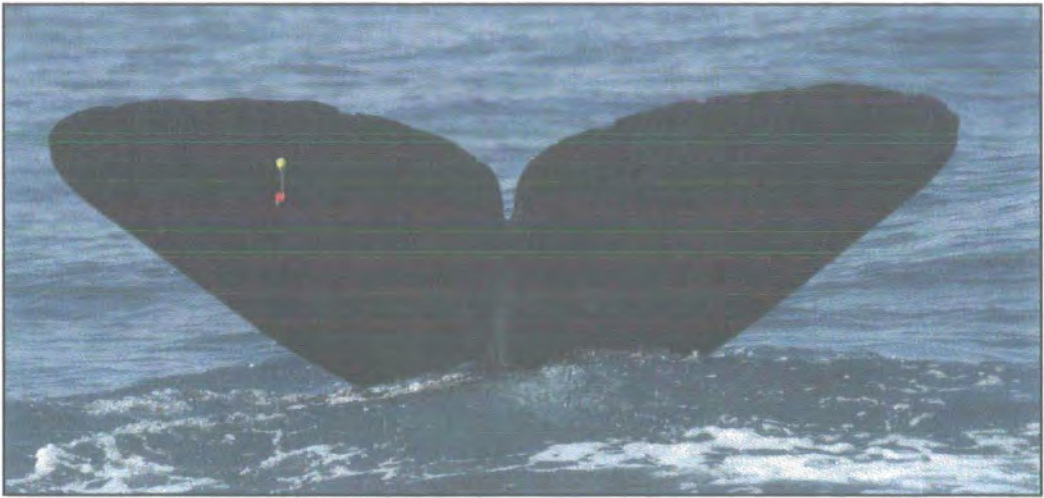




**Figure II.1** Geographic locations included in the study. Samples were primarily obtained from whales found within areas A and B in the Gulf of Mexico (GOM); areas C, D and E in the Mediterranean Sea (MED); areas F, G, H and I in the North Sea (NSEA) and areas J, K, L, M, N, O and P in the North Atlantic Ocean (NAO).

and sloughed skin ( $n=43$ ) samples from free-ranging sperm whales located in the GOM were collected under the National Marine Fisheries Service Marine Mammal Protection Act (MMPA) / Endangered Species Act (ESA) permit no. 909-1465-01 issued to Daniel Engelhaupt during five separate Minerals Management Service (MMS) sponsored cruises. Forty-nine samples, consisting primarily of sloughed skin ( $n=45$ ) and biopsy samples ( $n=4$ ) from sperm whales seen in the MED, were provided by the following researchers: Sabina Airoidi, Ana Canadas, Alexandros Frantzis, Mark Johnson, Patrick Miller and Renaud de Stephanis. Targeted whales were approached from the side-rear to avoid any startle reaction associated with approaching in the whale's 'blind-spot' (directly behind the whale). Biopsy samples were taken from the underside of the tail flukes (Figure II.2) or below and behind the dorsal hump (Figure II.3) by an ethanol sterilized stainless steel cylindrical dart tip (6 mm in diameter X 2 cm long) attached to a carbon shaft dart with independent floatation instead of a tether to avoid the possibility of entanglement. A .22 calibre dart rifle and a Barnett 150 lb draw weight crossbow were used to propel the darts. The skin sample is retained by three hooks at the centre of the dart tip, collected free floating and preserved in a salt saturated 20% dimethylsulfoxide (DMSO) solution (Amos and Hoelzel 1991) until further analysis could take place at the University of Durham's Department of Biological Sciences laboratory. Biopsy sampling has been





**Figure II.2** Tail fluke biopsy sampling. Characteristic markings on the tail flukes used to identify individuals. The biopsy dart is visible on the left side of the image as it contacts the left lobe of the whale's tail flukes.



**Figure II.3** Dorsal hump biopsy sampling. Dart with corer tip is visible to the left of the dorsal hump.

successfully performed on a number of different cetacean species and behavioural impact results show minimal effects to the animals. In accordance with MMPA/ESA permit regulations, immediate surface behavioural reactions to biopsy sampling were recorded and those results are discussed in detail in Chapter 3.

Several species of cetaceans, including sperm whales, are known to naturally slough their outer layer of skin (Amos et al. 1992). On occasion, sperm whales will leave sloughed skin behind in the footprint after a 'fluke-up' dive (often indicative of a deep foraging dive), during heavy bouts of social activity with other whales or following aerial activity events such as breaching, lobtailing and tail-slapping. This free-floating skin can be quickly collected with a dip-net and preserved in solution for later analysis. Previous sperm whale genetic studies have incorporated DNA extracted from sloughed skin with reasonable success (Richard et al. 1996a, 1996b; Christal 1998; Lyrholm and Gyllenstein 1998; Bond 1999). While sloughed skin has proven itself as a relatively uninvasive means for gathering genetic data on free-ranging whales there are several problems that may occur with its use (Valsecchi et al. 1998; Bond 1999).

On 18 occasions, sloughed skin was opportunistically collected from the suction cups of the Woods Hole Oceanographic Institute's (WHOI) DTAG. The DTAG is designed to digitally record exposure levels of noise (with particular respect to sound emitted by seismic vessels). In addition, it records the sounds made by the tagged whale, depth, and orientation throughout a whale's multiple dive cycles. The combination of genetic information (i.e. gender and relatedness to other DTAG tagged whales) and DTAG results will provide valuable data on how male and female sperm whales react to anthropogenic noise in addition to how they forage at depth.

During 2002, satellite-monitored tags developed by Oregon State University (OSU) were attached to sperm whales to record movements and habitat use within the northern GOM. Tissue samples were collected via biopsy sampling from fourteen of the eighteen whales outfitted with these 'satellite tags'. The combination of genetics and satellite-monitored tags will not only provide insight into the movement patterns of males and females within the GOM, but how related and unrelated members of clusters and groups outfitted with these tags stay together or split apart through space and time.

When possible, photoID was taken of the sampled whale's tail flukes or other distinguishing body features for future identification purposes based on techniques described by Arnborn (1987) (see Figures II.2 and II.3). The trailing edge of a sperm whale's tail flukes may contain a variety of markings such as nicks, scallops, waves, tooth-rake scars, holes, colouration patterns, notches and missing pieces that allow individuals to be identified over extensive periods of time (Arnborn 1987; Whitehead 1990). Less frequent markings such as cookie-cutter shark bites, tooth-rake scars and pigmentation patterns on the dorsal surface of the body and nicks, notches, holes and calluses on or near a whale's dorsal hump were used as a means to identify whales over hours to days to reduce the possibility of re-sampling an individual. The sperm whale photoidentification effort is part of Charlotte Cates and Dr. Nathalie Jaquet's behaviour and site-fidelity focused research projects. Their results are to be combined with the genetic analyses as soon as their analyses are completed.

### **II.2.2 Stranded Whale Sampling**

Cetacean strandings may involve lone individuals or large groups of whales. Group strandings are often associated with species that appear to have a tight social cohesion such as sperm whales and pilot whales. Some researchers suggest that group strandings may be caused by a lead animal of a group becoming disoriented or ill and then leading the rest of the group to shore. Disorientation may be caused by a variety of factors such as fluctuations in magnetic fields, sudden tidal changes or massive auditory disturbances. Strandings may also be caused by fisheries interactions, pollution and natural mortality. Although tissue samples collected from stranded whales allow researchers to gather essential information from deepwater species that are otherwise relatively inaccessible, caution should always be taken when interpreting information from stranded samples due to factors (e.g. currents, tides, illness, etc.) that may be directly influencing the whale's eventual location on the beach. Thirty-one opportunistic samples of dead stranded sperm whales were taken by the Texas Marine Mammal Stranding Network (n=3), Hubbs Sea World Research Institute (n=1), the Caribbean Marine Mammal Stranding Network (n=3), Clearwater Marine Park (n=1), Alexandros Frantzis (n=3) and Bob Reid (Scottish Stranding Co-ordinator) (N=20) and were included in this study.

### II.2.3 Preservation and Labelling of Samples

The potential for cross contamination when handling tissue samples for DNA analyses is one that must be dealt with in the appropriate manner to avoid inaccurate and potentially misleading results. A new pair of latex gloves was used to handle each individual sample collected to minimize the risk of contaminating one whale's DNA with another's. Manufacturer sterilized disposable blades were used to subsample larger samples from strandings down to a usable size. Forceps, scissors and biopsy corer tips were cleaned in the field for re-use in the following manner: 1) scrub with degreasing detergent, 2) rinse with distilled water, 3) rinse with 10% bleach water, 3) rinse with distilled water, 4) rinse with 95% ethanol, 5) flame sterilize before use. Dipnets were vigorously rinsed before gathering individual pieces of sloughed skin.

Tissue samples were preserved in 1.5 ml autoclaved vials containing a 20% DMSO salt-saturated solution (Amos and Hoelzel 1991). The ability of the DMSO solution to preserve samples for long durations is extremely beneficial when working in an offshore environment. Sample vials were labelled with the date and number of sample taken that day (yy/mm/dd/individual number) as well as the location of the sampling event. For example, sample number 000716-01GOM was the first (-01) sample taken in the year 2000 (00) on July (07) sixteenth (16) from the GOM. Detailed sample sheets recorded important supplementary information for each individual sample.

Samples from the northern GOM, western Atlantic Ocean and the Caribbean Sea were transferred to the University of Durham under proper MMPA/ESA and Convention on International Trade in Endangered Species (CITES) permits. Upon arrival, samples were frozen in a -20°C freezer until DNA extraction, processing and analyses could occur.

### II.3 LENGTH ESTIMATES OF FREE-RANGING WHALES

Estimates of an individual whale's age (calf, immature and adult) were determined from visual estimates of overall length determined from the small boat biopsy crew during a sampling event and compared to those described by Best (1979). Lengths from whales stranded on the beach were measured from the tip of the flukes to the tip of the head using a standard tape measurer.

## II.4 GROUP SIZE ESTIMATES OF FREE-RANGING WHALES

Group size estimates were determined from visual observations based from the 'flying bridge' of the primary research vessel using 25 X 150 'big-eye' binoculars, 10 X 50 hand held binoculars and acoustic listening using a towed five-element hydrophone array. Unfortunately, we were unable to maintain contact with the same group over extended periods of time. As a result, a new group was started each day even though we may have been in the same general vicinity as a previously sampled group.

## II.5 MOLECULAR GENETIC TECHNIQUES

### II.5.1 DNA Extraction

Whole-cell DNA was extracted for use in Polymerase Chain Reactions (PCR) by utilizing the phenol/chloroform extraction technique described in Hoelzel (1998a). A small portion of tissue (approximately 3-5 mm in width) was placed in an autoclaved Eppendorf<sup>®</sup> tube containing 500 µl of digestion buffer (100 mM NaCl, 10 mM Tris-HCl pH 8.0, 20 mM EDTA, 2% (w/v) sodium dodecyl sulfate (SDS); stored at room temperature) and was finely chopped using sterilized scissors. Thirty µl of proteinase K solution (10 mg/ml proteinase K in H<sub>2</sub>O) was added to the tubes and incubated at 37°C overnight to break down proteins. Five hundred µl of phenol was added to the solution and mixed moderately for three minutes. The mixture is centrifuged for 1 minute at 13,000 rpm using a Hettich EBA 12 centrifuge. The aqueous phase was collected and the previous two steps were repeated using 500 µl of phenol/chloroform/isoamyl alcohol (25:24:1), followed by 500 µl of chloroform/isoamyl alcohol (24:1). The aqueous phase was mixed with 1/10 the volume (approx. 50 µl) 3 M sodium acetate and 1 ml of chilled 100% ethanol. The tube was gently mixed and left at room temperature for 20 minutes if a precipitate was visible. If no precipitate was visible, as was generally the case with all sloughed skin tissue, then the tube was stored at -20°C for one hour before proceeding further. The tube was centrifuged for 20 minutes at 13,000 rpm and the supernatant was carefully poured off leaving behind a pellet. The previous step is repeated with 1 ml of chilled 70% ethanol. After removing the supernatant, the pellet was dried under a vacuum and dissolved in an adequate volume (200-500 µl dependent on the

pellet size) of 1X TE (10 mM Tris-HCl pH 7.6, 1 mM EDTA pH 8.0; autoclaved, filtered, and stored at room temperature) at 65°C for ten minutes then stored long-term at -20°C.

DNA quantity and quality were determined using electrophoresis techniques. Five µl of extracted DNA were combined with 2 µl of loading buffer (30% glycerol, 0.25% bromophenol blue, 0.25% xylene cyanol FF) and 5 µl of water before being loaded into separate wells of a 0.8 – 1.2 % agarose 0.5 x TBE (Tris-borate, EDTA) gel containing 2 drops of a 50 µg/ml ethidium bromide solution to allow for DNA visualization. A DNA 1 Kb (1000 bp) ladder (Sigma-Aldrich Inc.) was used as a molecular size marker to determine approximate quantities of DNA. Gels were run at 100 V in 0.5 x TBE buffer for 35 minutes on Bio Rad – Wide Mini-Sub™ Cell and Mini-Sub™ Cell GT machines. Final results were visualized using a Bio Rad Gel Doc 2000 with Quantity One 4.0.3 software. Those samples that did not provide a sufficient amount of DNA to work with were subsequently removed from further processing.

## II.5.2 Microsatellite Loci

### II.5.2.1 Selection of microsatellite loci

Microsatellite loci primers were selected based on published allele size ranges, levels of polymorphism and the visualization of ‘clean’ amplification product bands on an agarose gel to maximize the efficiency of using ABI techniques. The incorporation of pre-developed microsatellite primers isolated for several species of cetaceans (including bottlenose dolphins (*Tursiops truncatus*), beluga whales (*Delphinapterus leucas*), humpback whales (*Megaptera novaeangliae*), minke whales (*Balaenoptera acutorostrata*), blue whales (*Balaenoptera musculus*), fin whales (*Balaenoptera physalus*) and sperm whales) allowed for both a comparison with and an addition of data to previously published results. The majority of the loci selected were dinucleotide repeats. Two loci tested, GATA28 and GATA417, are tetra-nucleotide repeats which can lead to easier scoring due to a reduction in stutter bands produced (described later).

### II.5.2.2 Polymerase Chain Reaction (PCR) amplification and optimization

Amplification of 33 previously published microsatellite primer sets was attempted in separate 15 µl PCR reactions with the following standard conditions: 10 mM Tris HCl, pH 8.4, 500 mM KCl, 1.0 to 2.0 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 0.25µM of each primer, 0.3 units of Taq, and 10-100 ng/µl of template DNA. All amplifications were performed on either a Hybaid PCREXPRESS™ or MJ Research, INC PTC-100™ PCR thermocycler. Loci were titrated for optimization by adjusting the annealing temperature, number of cycles and amount of MgCl<sub>2</sub> involved in the reaction to establish proper amplification reaction conditions. Two thermocycling profiles (denoted as PM1 and PM2) were utilized for all loci tested. The PM1 profile consisted of an initial denaturing step of 95°C for five minutes, 35 cycles (30 seconds (s) at 95°C, 60 s at specified annealing temp, 60 s at 72°C) followed by a final extension step of eight minutes at 72°C. The PM2 profile consisted of an initial denaturing step of 95°C for five minutes, 35 cycles (60 seconds (s) at specified annealing temp, 60 s at 72°C, 40 s at 94°C) followed by one cycle at the specified annealing temp and a final extension step of five minutes at 72°C.

The resulting amplification products were visualized on a 1.0 – 1.2 % agarose gel (see above for gel preparation) to establish whether proper amplification had been achieved. A DNA 100 bp ladder (Sigma-Aldrich Inc.) was used as a molecular size marker to determine approximate sizes of PCR-fragments. Only loci with visible amplification products of the estimated correct size were included in additional processing.

After repeated attempts to amplify loci by altering amplification and optimization conditions, 17 loci (D14, D18, D28 – Shinohara et al. 1997; EV14, EV21, EV30, EV92 – Valsecchi and Amos 1996; FCB4, FCB6, FCB8 – Buchanan et al. 1996; GATA053 – Palsboll et al. 1997a; KWM2A, KWM1B, KWM2B, KWM12A – Hoelzel et al. 1998b; TEXVET3, TEXVET7 – Rooney et al. 1999) either failed to amplify the specific fragment, possessed additional fragment and erratic stutter ‘bands’ that could not be accurately read by Genotyper™ 2.0 (ABI) software or showed no significant level of polymorphism after screening a minimum of fifty individuals on Genotyper™ 2.0 software to be deemed beneficial in future analyses. These seventeen were consequently discarded. The remaining sixteen loci (EV1, EV5, EV37, EV94, EV104 – Valsecchi and Amos 1996; SW10, SW13, SW19

– Richard et al. 1996*b*; FCB1, FCB14, FCB17 – Buchanan et al. 1996; DO8, D22 – Shinohara et al. 1997; GATA28, GATA417 – Palsboll et al. 1997*a*; TEXVET5 – Rooney et al. 1999) were amplified using conditions described in Table II.1. Primer sequences and repeat type for each microsatellite are provided in Table II.2.

**Table II.1** PCR reaction conditions for the sixteen loci used.

Locus	Annealing Temp. (°C)	MgCl <sub>2</sub> (mM)	PCR Profile
EV1	57	1.5	PM1
EV5	59	1.5	PM1
EV37	56	1.5	PM2
EV94	55	1.0	PM1
EV104	54	1.0	PM1
SW10	56	1.5	PM1
SW13	57	2.0	PM1
SW19	56	1.5	PM1
DO8	52	1.0	PM2
D22	59	1.0	PM1
FCB1	53	1.5	PM1
FCB14	53	1.5	PM1
FCB17	56	1.0	PM1
TEXVET5	60	1.0	PM2
GATA28	53	1.5	PM1
GATA417	56	1.5	PM1



Table II.2 Locus, primer sequences and repeat type for each microsatellite. Microsatellite loci primer sequences are listed in the 5' to 3' direction.

Locus	5'	Primer Sequence	3'	Repeat Type
<b><u>EV1</u></b>				(AC) <sub>13</sub> (TC) <sub>8</sub>
Forward		CCC TGC TCC CCA TTC TC		
Reverse		ATA AAC TCT AAT ACA CTT CCT CCA AC		
<b><u>EV5</u></b>				(GC) <sub>2</sub> (GT) <sub>2</sub> (GC) <sub>11</sub>
Forward		AGC TCC CTT AGA CTC AAC CTC		
Reverse		TAT GGC GAG GGT TCC G		
<b><u>EV37</u></b>				(AC) <sub>24</sub>
Forward		AGC TTG ATT TGG AAG TCA TGA		
Reverse		TAG TAG AGC CGT GAT AAA GTG C		
<b><u>EV94</u></b>				(TC) <sub>6</sub> [...](AC) <sub>20</sub>
Forward		ATC GTA TTG GTC CTT TTC TGC		
Reverse		AAT AGA TAG TGA TGA TGA TTC ACA CC		
<b><u>EV104</u></b>				(AC) <sub>14</sub> (GCAC) <sub>2</sub>
Forward		TGG AGA TGA CAG GAT TTG GG		
Reverse		GGA ATT TTT ATT GTA ATG GGT CC		
<b><u>SW10</u></b>				(GTGC) <sub>7</sub> (GT) <sub>16</sub>
Forward		ACC TAA GGA TGG AGA TG		
Reverse		ATT TCC CAG GTC TGC AA		
<b><u>SW13</u></b>				(GT) <sub>20</sub>
Forward		AGC TGT CTT AAT GAA ATT CCC		
Reverse		ACG TAA ATG ATG CTG TT		
<b><u>SW19</u></b>				(AG) <sub>4</sub> (TG) <sub>26</sub>
Forward		GTA GTT TTC TTT AAC AGT AAT G		
Reverse		AGT TCT GGG CTT TTC ACC TA		
<b><u>D08</u></b>				(TG) <sub>18</sub>
Forward		GAT CCA TCA TAT TGT CAA GTT		
Reverse		TCC TGG GTG ATG AGT CTT C		
<b><u>D22</u></b>				(CA) <sub>3</sub> -TA-(CA) <sub>21</sub>
Forward		GGA AAT GCT CTG AGA AGG TC		
Reverse		CCA GAG CAC CTA TGT GGA C		
<b><u>FCB1</u></b>				(AC) <sub>16</sub>
Forward		TGC ATC TCC ATG GTA TGT CTT ATC C		
Reverse		AGC CTC TGC TAT GCC TGG AAC GC		
<b><u>FCB14</u></b>				(CT) <sub>11</sub> (TTCT) <sub>2</sub> (CT) <sub>8</sub>
Forward		CTA CAT TTG CCT CTT ATA GAC ATA GC		
Reverse		AAG TTG TCT TAG TTA GTC TGT GCT C		
<b><u>FCB17</u></b>				(TG) <sub>28</sub>
Forward		TCA GCC TCT ATA ACG TCC TGA GC		
Reverse		ATG GGG ACT GCC TAT ATT AGT CAG		
<b><u>TEXVET5</u></b>				(CA) <sub>24</sub>
Forward		GAT TGT GCA AAT GGA GAC A		
Reverse		TTG AGA TGA CTC CTG TGG G		
<b><u>GATA028</u></b>				(GATA) <sub>4</sub>
Forward		AAA GAC TGA GAT CTA TAG TTA		
Reverse		CGC TGA TAG ATT AGT CTA GG		
<b><u>GATA417</u></b>				(GATA) <sub>14</sub>
Forward		CTG AGA TAG CAG TTA CAT GGG		
Reverse		TCT GCT CAG GAA ATT TTC AAG		

### **II.5.3 Microsatellite Allele Scoring Using Automated Fluorescence**

#### **II.5.3.1 *Pre-ABI electrophoresis***

Upon achieving primer optimization conditions, microsatellite loci were PCR-amplified with fluorescently labelled FAM, HEX or NED ABI dyed (Perkin-Elmer Inc.) forward (5'-3') primers. The use of labelled primers allows for subsequent visualization of the PCR product on a sequencing gel with the ABI system (ABI Biosystems<sup>TM</sup>, Foster City, California). Fifteen µl PCR reactions (using previously described reaction conditions) included 10-20% (0.025µM) labelled forward primer and 80% non-labelled forward primer. The use of 100% labelled primer may cause spikes (sharp peaks off the readable scale) on the GeneScan<sup>TM</sup> gel that are both complicated to read and may overlap with other dye colours on GeneScan<sup>TM</sup> and Genotyper<sup>TM</sup> 2.0 software. The use of agarose gel electrophoresis allowed amplification products to be distinguished on a 1.0 – 1.2 % agarose gel before running the product on the sequencer. Only visible products of the expected size were subsequently run on the ABI sequencing gel.

Loci and corresponding fluorescent dyes were configured to avoid overlap in size ranges. PCR products whose alleles did not overlap in estimated size (bp) and products that contained different dyes (FAM, HEX, NED) were loaded and run in the same lane to increase the efficacy of data collected from an individual ABI gel. If two loci could potentially overlap in range, one would be labelled with a different dye to avoid difficulty in the interpretation of allele size results. This allowed for maximization of the gel's ability to screen numerous labelled PCR-products at once. For each locus used, a labelled sample run on a previous ABI gel was added as a means of control for allele size consistency among gels.

#### **II.5.3.2 *ABI electrophoresis***

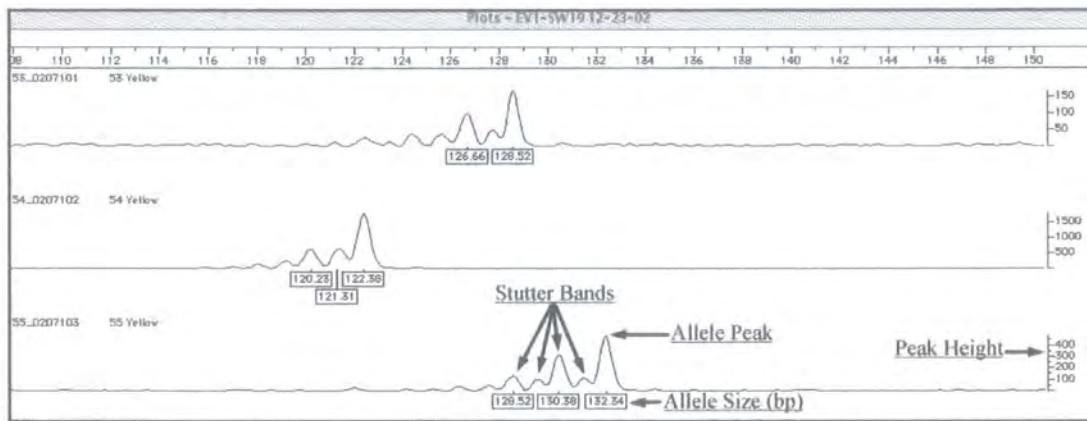
ABI 373 and 377A automated DNA sequencers were used to run labelled PCR products on a 50 well 6% polyacrylamide denaturing sequencing gel. Labelled PCR-products were prepared for Genescan<sup>TM</sup> Analysis 2.0 software analysis by adding specific amounts of the product (0.25 µl for FAM labelled products, 0.30 µl for HEX and 0.40 µl for NED) to a 1.625 µl mixture of ABI loading buffer containing the ROX-500 internal size standard for each lane. Gels were run for 3 hours at 41°C on the automated sequencer. The products are passed through a set

matrix and scanned by a laser at regular intervals as they run off the gel. The number of scans the laser collects when detecting a peak is converted to base pairs by way of an internal size standard dye (ROX 500 ladder) previously loaded in each lane with the PCR products. Not only are microsatellite peaks detected, but additional peaks such as ‘stutter bands’ (several small peaks 1-2 bp apart depending on the repeat and just before the tallest peak) and non-specific PCR amplification ‘stray bands’ are recognized as well. Stutter bands appear to be more common in dinucleotide repeats than in tri- and tetra-nucleotide repeats and can be quite characteristic and helpful in identifying microsatellite alleles (Schlotterer 1998). Stray bands may pose problems in the interpretation of allele size results, but can often be eliminated based on their poor structure.

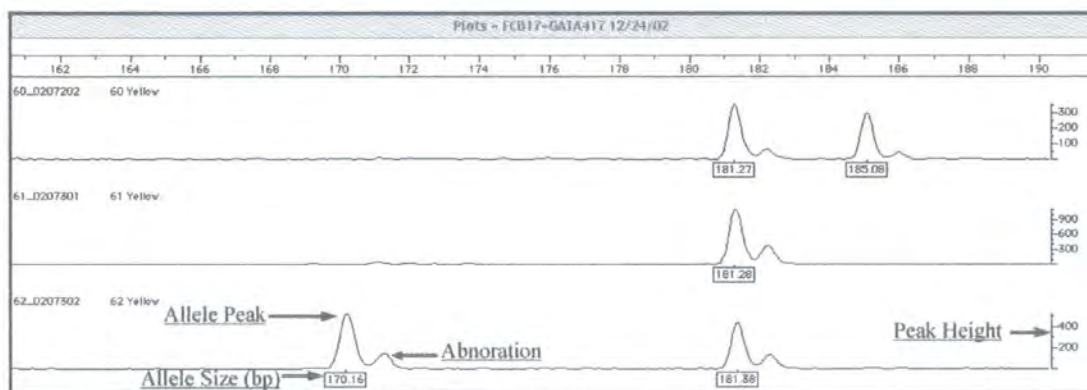
GeneScan™ Analysis 2.0 software (ABI) for Macintosh was used to interpret and analyze the raw data collected from the ABI sequencing gels. The ROX-500 internal size standard ladder peaks are defined at 35, 50, 70, 100, 139, 150, 160, 200, 250, 300, 340, 350, 400, 450, and 500 bp. When visualizing the finished gel, FAM labelled products = blue, HEX = green and NED = yellow. The labelled product peak (allele) sizes are estimated based on their migration rate compared to the migration of the known ROX-500 standard. This allows for proper scoring (determination of DNA allele sizes in bp) of the labelled DNA products.

### **II.5.3.3 Genotyper analysis**

GeneScan™ 2.0 data files were imported into the ABI analysis program Genotyper™ 2.0 for interpretation of allele sizes. Each individual dye colour is plotted against the internal size standard ROX-500 scale, allowing for multiple lanes from a colour to be viewed together. Peaks (allele sizes) are selected based on their intensity (height) and structure according to the user’s parameters. As previously described, an allele’s structure may or may not include accompanying ‘stutter’ bands depending on the microsatellite’s repeat motif. Figure II.4 shows examples of both dinucleotide (with preceding stutter bands spaced approximately 1 bp apart) and tetranucleotide repeat microsatellite allele as visualized with Genotyper™ 2.0 software. Tetra-nucleotide microsatellite alleles have reduced stutter bands that can lead to easier scoring (Tautz and Schlotterer 1994; Palsboll et al. 1997a). Allele



a) Dinucleotide repeat microsatellite. Stutter bands precede the actual microsatellite allele.



b) Tetranucleotide repeat microsatellite. No stutter bands are visible before the allele peak.

**Figure II.4** Genotyper<sup>TM</sup> file depicting allele structures for a) dinucleotide (with visible stutter bands) and b) tetranucleotide repeat microsatellite loci. Allele sizes (bp) are depicted in the scale across the top of the figure and in the boxes under the allele peaks and peak height is seen on the far right of the figure.

sizes may fluctuate slightly, resulting in a slight discrepancy in labelled sizes between gel runs because the allele sizes are not integers. As a result, minute differences in allele size (ex. 0.4 bp) were discarded, scored as the same allele, and expressed as an integer number for classification (see David and Menotti-Raymond 1998 for additional details on scoring). Alleles with low peak heights (< 80) or poor structure were often noted and discarded from further analysis as they appear to 'slip' a few base pairs from their true size. These poor quality scans for alleles were often associated with degraded and/or low quantity DNA samples extracted from sloughed skin. Poor quality scan results would be re-amplified and run on the sequencing gel until either a size could be determined or they were deemed

ineffective at that particular locus. Due to potential allele size shifts across gels, previously run control samples were included to ensure allele size estimation consistency among gels. If the allele size estimation for the control sample differed between the two separate gels, then the samples were not incorporated into the overall results and were processed on a new ABI gel.

## **II.5.4 Mitochondrial DNA (mtDNA)**

### **II.5.4.1 *mtDNA* amplification**

An approximately 450 bp segment of the mtDNA control region HVR1, including the most variable 58-324 bp region identified by Lyrholm et al. (1996), was amplified using primers L15812 5'-CCTCCCTAAGACTCAAGG-3' (Arnason et al. 1993) and H16343 5'-CCTGAGAATGCAACTAGAGG-3' (Southern et al. 1988). The L15812 primer anneals to a portion of the tRNA threonine and the tRNA proline genes, while the H16343 primer anneals to the central conserved region. Amplification was carried out in separate 30 µl PCR reactions with the following conditions: 10 mM Tris HCl pH 8.4, 50 mM KCl, 1.3 mM MgCl<sub>2</sub>, 200 µM of each dNTP, 0.25 µM of each primer, 0.3 units of Taq polymerase and 10-100 ng of template DNA. The PCR thermocycling profile consisted of an initial denaturing step of 95°C for five minutes, 35 cycles (90 seconds (s) at 55°C, 90 s at 72°C, 45 s at 72°C), followed by one cycle of 55° for 90 s and a final extension step of eight min at 72°C.

PCR-amplification products were checked for efficacy by size-fractionation on a 1.2% agarose TBE gel (gel preparation previously described). Negative PCR controls (reaction volumes excluding DNA) were included to assure that all amplification reactions were free of cross-contamination.

### **II.5.4.2 *mtDNA* purification and sequencing**

QIAquick (QIAGEN, Valencia, California) PCR Purification Kits with spin columns were used to purify DNA fragments from excess primers and dNTPs. The purified product was subsequently run on a 1.2% agarose gel to determine DNA amounts (ng/µl). DNA sequencing reactions were performed using standard conditions: 25 cycles of 10 sec at 96°, 7 sec at 50°C and 4 min at 60°C on a Perkin Elmer GeneAmp PCR system 2400™. Amplified products were sequenced directly with Big Dye

termination chemistry on a 6% denaturing polyacrilamide DNA sequencing gel for fluorescent imaging on automated DNA sequencers, model numbers 373 and 377, using manufacturer (P.E. Biosystems, Wellesley, Massachusetts) protocols. Resulting files, in the form of DNA sequences and electropherograms, provide the nucleotide and its peak defined by the ABI sequencer. These files are then imported into the Sequencer<sup>TM</sup> version 4.1.2 software program (available from the Gene Codes Corp. at <http://www.genecodes.com>) where individual sequences and electropherograms are viewed for possible ambiguities (i.e. mistaken nucleotide assignments). Discrepancies are manually corrected by the user assigning the correct nucleotide based on the electropherogram peak within the sequence.

#### **II.5.4.3 *mtDNA alignment***

Sequence alignment for multiple individuals was done automatically using Sequencer 4.1.2 software. Multiple sequence alignments and their corresponding chromatograms were edited by eye for discrepancies in base-calling (Figure II.5). From the 450 bp section of sequenced data, 399 bp were used in the analysis due to difficulties associated with interpreting the beginning and ending flanking regions. After sequence formatting, a 'contig', or consensus file, of all sequences can be exported and used for population analyses. Both strands (heavy and light chains) were sequenced for unique haplotypes as a means of verification.

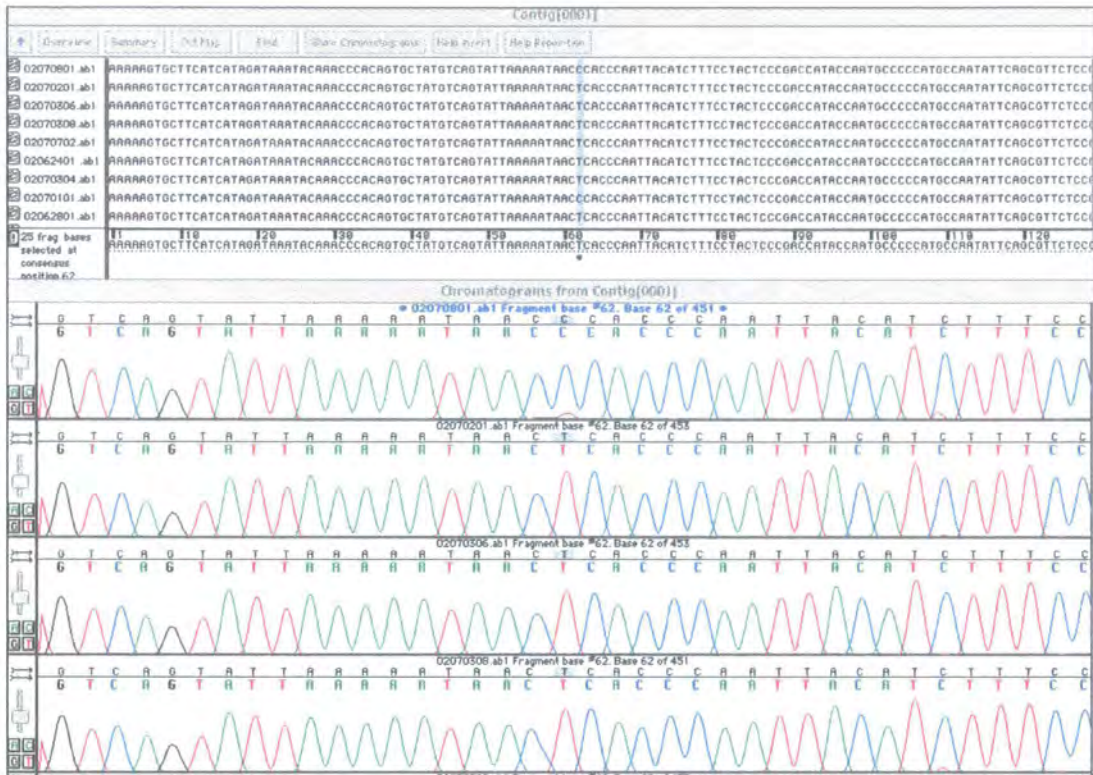
The resulting sequences were aligned with twenty-three sperm whale sequences provided by Dr. Sarah Mesnick (Southwest Fisheries Science Centre) using the program Mega 2.0 (<http://www.megasoftware.net/>) (Kumar et al. 1993). This combination of sequences includes all the haplotypes discovered to date that occur for sperm whales on a global scale.

## **II.6 GENETIC ANALYSES**

### **II.6.1 Identification of Individuals**

The potential for re-sampling a previously sampled whale during the study was a concern due to the low minimum population estimate in the northern GOM, the fact that female sperm whales tend to exhibit philopatry to particular geographic areas and multiple field seasons being focused in specific geographic areas (i.e. the





**Figure II.5** Sequence alignment and chromatograms as visualized through Sequencher™ software. Individual sequences are seen in the top half of the figure and the chromatograms that depict base peaks for each of those sequences are visualized in the lower half of the figure. Discrepancies in base calling are visualized via the accompanying chromatogram.

Mississippi River Canyon). The program Cervus 2.0 (Marshall et al. 1998; available at: <http://helios.bto.ed.ac.uk/evolgen/cervus/cervus.html>) was used to check the identity of whales by finding matching genotypes within the dataset. Cervus records genotypes and IDs that occur more than once in the dataset file. Duplicate genotypes (i.e. tissue samples from the same whale) were subsequently discarded from the datasets used for population and relatedness calculations, but were included as a means of providing re-sighting data over time.

### II.6.1.1 Probability of identity

To avoid biases associated with the unintentional inclusion of multiple samples from the same individual whale, genotype screening at six of the least polymorphic microsatellite loci (EV1, EV5, EV104, SW10, GATA28 and GATA417) was performed on all collected samples. Samples were considered genetic duplicates if

they matched at all six loci. The rationale for this approach is that the probability of randomly sampling two whales containing the same genotypes across multiple polymorphic loci is extremely low. Probability of identity estimates are based on population allele frequencies and the number of alleles at each locus. The estimate was calculated using the following formulae described by Paetkau and Strobeck (1994):

$$\sum_i p_i^4 + \sum_i \sum_{j>i} (2p_i p_j)^2$$

where  $p_i$  and  $p_j$  are the frequencies of the  $i$ th and  $j$ th alleles.

### II.6.2 Molecular Sexing

Although sperm whales are considered the most sexually dimorphic of the cetaceans with males growing to lengths of 18.3 m and nearly 57 tons and females reaching up to 12.5 m and approximately 24 tons (Best 1979; Rice 1989), the accurate determination of cetacean gender at sea based on physical characteristics can be both difficult and unreliable (Christal 1998; Gowans et al. 2000). The presence or absence of calluses (a roughened patch on the dorsal hump) is thought to be a secondary sexual characteristic primarily (73%) associated with females taken during whaling operations (Kasuya and Ohsumi 1966). However, calluses were also present on up to 30% of immature males taken by whalers. Recent genetic findings by Christal (1998) also imply that the presence of calluses is not sex specific. For these reasons, the presence or absence of calluses should not be considered a reliable method for gender determination in the field.

Advances in molecular sexing techniques, focusing on the Y chromosome's ZFY gene, allow us to elevate the level of information taken from biopsy and sloughed skin samples collected from cetaceans in the wild. The incorporation of the ZFY/ZFX molecular sexing technique (Berube and Palsboll 1996) permits us to advance our understanding of both social and population structure issues by incorporating gender into the equation.



### II.6.2.1 ZFY/ZFX technique

Gender determination was performed using odontocete-specific primers (ZFYX0582F, ZFY0767F, and ZFX0923R) that amplify the ZFX and the ZFY sequence (Berube and Palsboll 1996). All sperm whale samples were tested (including strandings) to determine and confirm gender using the techniques described above. Amplification of the ZFX and ZFY fragments were carried out in 20 PCR  $\mu$ l reactions with the following conditions: 67 mM Tris-HCl, pH 8.8, 2.0 mM MgCl<sub>2</sub>, 16.6 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 10 mM  $\beta$ -mercaptoethanol, 200  $\mu$ M of each dNTP, 0.5  $\mu$ M of each primer, 0.4 units of Taq<sup>TM</sup> DNA polymerase and 100 ng of template DNA. Amplification reactions were run on a MJ Research, INC. PTC-100<sup>TM</sup> programmable thermal controller with an initial 5-min denaturing step at 94°C, 37 cycles (60 s at 94°C, 60 s at 52°C, 60 s at 72°C) followed by a 5-min final extension at 72°C. Amplification products were distinguished on a 1.2% Agarose gel and scored against a DNA 100 bp ladder (Sigma). Females were defined as having only one band at approximately 383 bp, while males possessed a characteristic 227 base pairs (bp) fragment in addition to the 383 bp fragment. It is this 383 bp fragment that acts as a positive control verifying that the amplification reaction has taken place. Four controls, consisting of two confirmed male and two confirmed female samples taken from stranding events in the NSEA and GOM respectively, were run with each set of unknown samples to insure that both proper amplification had occurred as well as to provide a means of gender verification. A negative control, composed of the stock solutions only, was run to check for potential cross-contamination. Each individual sample was molecularly sexed using the ZFY/ZFX method a minimum of two times to verify the results.

### II.6.3 Eliminating Close Relatives – Background Allele Frequency Determination

Due to what appears to be an intricate level of social structure in sperm whales (Richard et al. 1996a; Christal 1998; Christal and Whitehead 2000; Whitehead et al. 1991), the main hurdle associated with examining questions of population structure and kinship is the determination of an accurate set of background allele frequencies to base unbiased heterogeneity and relatedness calculations on. Previous studies have noted that these female dominated sperm whale ‘mixed’ sex social groups exhibit an apparently high degree of philopatry to geographic locations and are

therefore likely to contain close relatives (e.g. mother-calf pairs) among group members (Richard et al. 1996a; Christal 1998; Lyrholm et al. 1999; Whitehead and Weilgart 2000). The incorporation of every individual's genotype within the population risks biasing results due to genetically similar (i.e. highly-related) individuals contributing to the calculation of background allele frequencies used in both types of analyses. The inclusion of relatives into statistical estimates of population structure violates the assumption that samples are independent of each other. An additional consequence is that relatedness values based on a putative population's allele frequencies may be misleading. However, if one were to assume that all members within a group are related and we were to eliminate all but one member of that group for population analyses (e.g. to determine allele frequencies), the result would yield very small sample sizes and thereby distort background allele frequencies.

To avoid biases associated with the inclusion of related whales for determining heterogeneity among putative populations as well as more realistic relatedness values, close relatives were removed from genotype sets used to establish background population allele frequencies. This reduction of close kin was done via a series of 5 steps:

1. All individuals in the given 'geographic' population (i.e. the GOM) were run through GENEPOP v. 3.2 (Raymond and Rousset 1995 – available at <http://www.cefe.cnrs-mop.fr/wwwsite/default-English.htm>) to determine a preliminary set of allele frequencies for each of 16 microsatellites. Although these values may be biased by the inclusion of close kin, this was merely a starting point to base further calculations on.
2. All individuals within the putative population were run through the Relatedness 5.0.7 program (Goodnight Software – available at <http://gsoft.smu.edu/GSoft.html>) to determine relatedness (R) values (see estimating relatedness below). Close relatives (pairs with R-values on the order of first order relatives ( $R \geq 0.30$  to be conservative) were removed from the dataset before additional processing through the Southwest Fisheries Science Centre's (SWFSC) Turbo Basic Kinbegone 1.3.1 program.

3. By basing calculations on the previously determined background allele frequencies established in steps 1 and 2, Kinbegone 1.3.1 was used to eliminate close relatives from the geographic populations. Kinbegone 1.3.1 allows for nine microsatellite loci to be used in the calculations. The program allows the user to set a threshold value ( $R \geq 0.40$  for our dataset) above which all individuals will be removed from the group. The program then finds the most highly related pair of individuals and removes the individual that contributes the most to the mean relatedness of the group. The procedure comes to an end when no pairwise relatedness values surpass the threshold value set by the user. The remaining set of individuals should constitute a relatively unrelated 'population' of whales from which background allele frequencies for all sixteen microsatellite loci can be accurately based.
4. The genotypes of the remaining unrelated individuals were then run through GENEPOP to determine a more accurate set of background allele frequencies that relatedness values can be based upon.
5. Data files for the programs Relatedness 5.0.7 and Kinship 1.3.1 (Goodnight Software – available at <http://gsoft.smu.edu/GSoft.html>) were constructed for individual groups, clusters and the population as a whole. Population allele frequencies, based on the '*restricted*' data set were included in all data files. As a means of allele frequency determination comparisons, I've constructed an 'all' data set that allows the Relatedness 5.0.7 program to calculate allele frequencies based on all individuals from the entire population. This should provide a meaningful comparison between 'all' and '*restricted*' datasets.

For thoroughness, two sets of data referred to as 'all' and '*restricted*' were used for population and relatedness analyses. The all dataset consisted of every individual within a particular geographic area (i.e. GOM, MED and the NSEA). The *restricted* dataset consisted of non-highly related individuals within a particular geographic area determined via the method described above.

### II.6.4 Relatedness and Kinship Assessments

Levels of genetic relatedness were calculated for pairs of individuals within clusters and groups using the programs Relatedness 5.0.2 and Kinship 1.3.1 according to Queller and Goodnight's (1989) method. A total of 96 whales from 25 groups (with 12 clusters imbedded within 9 groups) in the GOM and 17 whales from two group stranding events ( $n=11$  and  $n=6$ ) in the NSEA along the Scottish coast were included in relatedness and kinship calculations. Relatedness 5.0.2 and Kinship 1.3.1 were used to estimate Grafen's relatedness coefficient ( $R$ ) between all pairs of individuals based on the number of shared microsatellite alleles and population allele frequencies using a regression measure of relatedness:

$$R = \frac{\sum_x \sum_k \sum_l (P_{xyl} - P^*)}{\sum_x \sum_k \sum_l (P_{xyl} - P^*)}$$

where  $x$  indexes individuals in the data set,  $k$  indexes loci and  $l$  indexes allelic position (i.e.  $l = 1$  or  $2$  for a diploid individual,  $1$  only for a haploid).  $P_x$  and  $P_y$  are the frequency of the alleles within the current  $x$  and  $y$  individuals respectively.  $P^*$  is the frequency of the allele in the overall population (excluding all putative relatives of  $x$ ) – determined from methods previously described.  $R$ -estimates are based on background population allele frequencies. As a result,  $R$ -values may be biased as common alleles are more likely to be shared by chance than descent. Kinship 1.3.1 is able to compensate for this type of bias by assigning lower  $R$ -values to individuals that share common alleles and higher  $R$ -values to those that share rare alleles. Relatedness values were based on background allele frequencies with highly related ( $R \geq 0.40$ ) whales previously removed from the dataset (see above description of removing related whales from background allele frequencies). Relatedness measurements ranged from  $-1.0$  to  $+1.0$  with positive values signifying two individuals sharing more alleles that were identical by descent than expected by chance, whereas negative  $R$ -values were indicative of two individuals sharing fewer such alleles than expected by chance. When populations are in Hardy-Weinberg equilibrium, relatedness coefficients should average  $0.50$  for first-order relatives (e.g. parent-offspring and full-sibling pairs),  $0.25$  for second-order relatives (e.g. half-sibs, grandparent-grandchild, aunt/uncle-niece/nephew) and  $0.00$  for pairs of randomly chosen individuals that are not related.

Kinship tests hypotheses of pedigree relationships between pairs of individuals by calculating a likelihood ratio that a pair of genotypes fits a particular hypothesized relationship (Goodnight and Queller 1999). The likelihood ratio is based on R-values, population allele frequencies and the pair's genotypes. For example, if we want to examine whether a pair of individuals with an R-value of 0.50 is a first-order relation, we set our null hypotheses (no relation) to zero and our primary hypothesis (first-order relation) to  $R=0.50$ . Log likelihood values are calculated as Kinship performs a simulation routine (set at 10,000 repeats) to determine a distribution of log likelihoods and significance levels for likelihood ratios using the hypothesis settings and the population allele frequencies. Rejection or non-rejection of the null and primary hypotheses was determined based on the resulting significance levels.

Non-parametric randomisation tests using the Wilcoxon-Mann-Whitney  $U$  statistic in the STATXACT 6.0 program were performed to test for significant differences in mean relatedness values for whales found within and between groups and clusters. Associated  $p$ -values were based on a Monte Carlo resampling size of 20,000 and a 99% confidence limit.

## **II.6.5 Population Genetic Structure**

### **II.6.5.1 *Microsatellite loci analysis***

After the elimination of duplicate samples, individuals from the GOM ( $n=83$ ), MED ( $n=22$ ) and the NSEA ( $n=20$ ) were screened for all 16 polymorphic microsatellite loci. The resulting genotypes were used to provide an analysis of population structure based on bi-parentally inherited nuclear DNA.

#### **II.6.5.1.1 Population variation and levels of genetic diversity**

Levels of variation at microsatellite loci can be affected by migration, mutation, genetic drift and selection at linked loci. The state in which genotype frequencies in a population match the expectations of the Hardy-Weinberg law (equilibrium state of a single locus in a randomly mating diploid population that is free of migration, mutation, genetic drift and selection at linked loci) is the concept of Hardy-Weinberg equilibrium (HWE) (Gillespie 1998). When population samples show deviations

from HWE, it may be a result of factors such as physical linkage (an association between two or more genes as a result of their location on the same chromosome), inbreeding (i.e. non-random mating), non-random sampling of panmictic individuals from a large population or the Wahlund effect. Generally, inbreeding and population subdivision will cause an increase in the number of homozygotes within a population while outbreeding will cause an increase in heterozygosity levels (Gillespie 1998). However, an excess of observed homozygotes within the population may also be caused by the presence of null alleles (a non-amplifying allele (Callen et al. 1993)) or the Wahlund effect (a deficiency in the number of heterozygotes in subdivided populations relative to HW expectations based on a single large population (Robertson and Hill 1984)). Under HWE, observed and expected levels of heterozygosity should be relatively close in value. The departure of genotype frequencies from the predictions of HWE may imply the effect of selection.

Genetic diversity as a measure of individual variation within a population reflects the number of different types in the population, taking into account their frequencies (Gregorius 1987). Levels of genetic diversity for each population were examined using the computer program GENEPOP v. 3.2 (Raymond and Rousset 1995). The number of unique alleles was calculated for each locus and over all loci and the observed heterozygosity ( $H_O$ ) and expected heterozygosity ( $H_E$ ) levels at each locus were tested for deviation from HWE (null hypothesis: random union of gametes) using Fisher's exact test in the GENEPOP 3.2 program (Guo and Thompson 1992). GENEPOP 3.2 tests the probability of Fisher's exact test using the Markov chain method with 1000 dememorizations, 100 batches and 1000 iterations. The unbiased expected heterozygosity levels at each locus in every population were estimated as:

$$H_E = \frac{2n(1 - \sum p_i^2)}{(2n - 1)}$$

where  $p_i$  is the frequency of each of the alleles at a locus and  $n$  is the number of sampled individuals (Nei 1987; p. 178, eqn 84). The mean observed and expected heterozygosity were estimated by averaging across all loci.

### II.6.5.1.1.1 Allelic richness

The number of alleles found within a given population sample is highly dependent on a population's sample size. Allelic richness for each locus and for each population ( $R_s$ ) was measured to insure that the observed number of alleles found within a population is independent of a population's sample size. The concept is to estimate the expected number of alleles in a sub-sample of  $2n$  genes, given that  $2N$  genes have been sampled ( $N \geq n$ ). The smallest number of individuals typed for a locus in a sample is fixed as  $n$  and  $R_s$  is calculated with the program FSTAT 2.9.3 (Goudet 2001 – available at <http://www.unil.ch/izea/software/fstat.html>) as:

$$R_s = \sum_{i=1}^{n_i} \left[ 1 - \frac{\binom{2N - N_i}{2n}}{\binom{2N}{2n}} \right]$$

where  $N_i$  is the number of alleles of type  $i$  among the  $2N$  genes. Each term under the sum corresponds to the probability of sampling allele  $i$  at least once in a  $2n$  sample size. When allele  $i$  is so common that we are sure to sample it, then the ratio is undefined, but the probability of sampling the allele is set to 1.

### II.6.5.1.1.2 Null alleles

Null alleles are caused by mutations in one or both of the primer binding sites and result in the absence of an amplified PCR product (Callen et al. 1993). The use of several loci together may help to circumvent the problems caused by null alleles. The frequency of putative null alleles for all 16 loci/population was tested using the computer program CERVUS 2.0 (Marshall et al. 1998). CERVUS 2.0 uses an iterative algorithm that is based on the difference between the observed and expected frequency of homozygotes. Positive results may be explained by either null alleles causing an excess of homozygotes within the dataset or the presence of inbreeding within the population.

#### II.6.5.1.1.3 Linkage disequilibrium

A test for linkage disequilibrium (null hypothesis: independence between genotypes at separate loci) was completed for each pair of loci using GENEPOP 3.2 (Raymond and Rousset 1995) to determine whether associations existed between pairs of alleles. Linkage disequilibrium is brought about via factors such as natural selection, non-random mating and the presence of population substructure or mutation. Loci do not have to be on the same chromosome for linkage disequilibrium to occur. GENEPOP 3.2 creates contingency tables for all pairs of loci in each sample, then performs a probability test (Fisher's exact test) using a Markov chain approach (Guo and Thompson 1992). One thousand dememorizations, 100 batches and 1000 iterations per batch were used for each population.

#### II.6.5.1.1.4 Random mating assessment

Wright (1951) devised methods to test the genetic population structure in terms of three 'hierarchical'  $F$ -statistics ( $F_{IS}$ ,  $F_{IT}$  and  $F_{ST}$ ), or allelic correlations, used to distinguish three possible levels of inbreeding and to quantify population substructure. Wright's  $F_{IS}$  is the correlation between homologous alleles among individuals that are part of a local population (after Avise 1994). Non-random mating (e.g. mating with relatives) is one cause of the reduction in the heterozygosity of an individual within a subpopulation and was calculated using FSTAT 2.9.3 software. The degree of non-random mating (inbreeding) within a population was assessed by comparing the observed and expected heterozygosity levels using the following formulae:

$$F_{IS} = \frac{H_s - H_i}{H_s}$$

where  $H_i$  is equal to the observed heterozygosity of an individual, estimated as the mean frequency of heterozygotes averaged over all subpopulations and  $H_s$  is the expected heterozygosity of an individual in a subpopulation, calculated separately for each subpopulation and then averaged.



### II.6.5.1.2 Population differentiation

The measurement of genetic differentiation (variation between two or more populations, demes or subpopulations) for microsatellite data was analyzed using the five statistical approaches described below.

#### II.6.5.1.2.1 Fisher's exact test

Comparisons of microsatellite allele frequency distributions at each locus and between geographic populations were evaluated with Fisher's exact test (Raymond and Rousset 1995) using the population differentiation method in the program GENEPOP 3.2. The null hypothesis is that allelic/genotypic distributions are homogeneous across populations. However, the population's sample size may greatly influence the resulting *p*-values.

#### II.6.5.1.2.2 $F_{ST}$ statistics

Estimates of Wright's fixation index,  $F_{ST}$  (Wright 1951; Weir and Cockerham 1984), were calculated with the ARLEQUIN 2.0 (Schneider et al. 2000 – available at <http://lgb.unige.ch/arlequin/software>) and FSTAT 2.9.3.2 computer programs. This measure assumes an infinite allele mutation model (IAM) in which every new mutation creates a novel allele (an assumption that may be violated with microsatellites). Microsatellite loci perhaps better fit the stepwise mutation model (SMM) described by Shriver et al 1993) where the majority of mutations involve the gain or loss of one or two repeat units.  $F_{ST}$  is based on the variance in allele frequencies to determine the level of population subdivision caused by a reduction in heterozygosity (Avisé 1994; Majerus et al. 1996). This parameter varies from 0 (no differentiation) to 1 (complete differentiation) and is estimated using pairwise population comparisons of the proportion of variance that accounts for between rather than within population differences (Nei 1973; Weir and Cockerham 1984).  $F_{ST}$  is defined as:

$$F_{ST} = \frac{(H_t - H_s)}{H_t}$$

where  $H_t$  and  $H_s$  are proportional to the expected heterozygosity of an individual in the whole and subpopulation respectively. When calculating  $H_t$ , it is assumed that all the samples were selected from one homogeneous randomly mating population.  $H_s$  is first calculated for each subpopulation and then averaged together.  $F_{ST}$  is dependent on the assumption that populations are maintained under the same conditions, they are derived from a common ancestor and that gene frequencies are at a state of equilibrium (Tufto et al. 1996). Mutational relationships among alleles are not considered. Statistical significance was tested by 10,000 permutations of the data.

Since  $F_{ST}$  statistics were originally developed for loci with only two alleles, modifications to  $F_{ST}$  methods were developed so that this technique could be utilised for many loci and multi-allelic data.  $\theta$ , Weir and Cockerham's (1984) analogue to  $F_{ST}$ , is defined as:

$$\theta = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_b^2 + \sigma_w^2}$$

where  $\sigma_a^2$  represents the among sample variance,  $\sigma_b^2$  is the variance between individuals, and  $\sigma_w^2$  is the within individual variance component. Values of  $\theta$  were tested using a non-parametric, permutation-based approach with the ARLEQUIN 2.0 program. If differentiation between putative populations is nonexistent, then members from each population should be able to be split and randomly assigned to new groups. This should generate  $F_{ST}$  values comparable to the original.

#### II.6.5.1.2.2.1 Sex-bias in dispersal

An assessment as to whether differences in female and male dispersal rates affect population structure (seen via comparisons between both sexes for  $F_{IS}$ ,  $F_{ST}$ ,  $H_O$ ,  $H_S$  (the within group gene diversity), mean assignment and variance assignment) with respect to co-dominant genetic markers was performed using the program FSTAT 2.9.3.2 (Goudet et al. 2002). Weir and Cockerham's (1984) estimator of  $F_{IS}$  and  $F_{ST}$  were used. Testing assumes that the species in question has non-overlapping generations where dispersal occurs at the juvenile stage (before reproduction) and that an individual is sampled post dispersal (Goudet et al. 2002). When comparing

allele frequencies between individuals of the dispersing sex and those of the more philopatric sex, a greater similarity is expected among the more dispersing sex (Goudet et al. 2002). As a result,  $F_{ST}$  and mean assignment values should be higher among the more philopatric sex (i.e. females in this case).  $F_{IS}$ ,  $H_S$  and the variance assignment should be higher in members of the dispersing sex (males in our case) (Goudet et al. 2002).

#### II.6.5.1.2.3 $R_{ST}$ and $Rho_{ST}$

$R_{ST}$  was calculated using the program RSTCALC 2.2 (Goodman 1997 – available at <http://helios.bto.ed.ac.uk/evolgen/rst/rst.html>) and used to determine population differentiation based on microsatellite allele frequencies (Slatkin 1995). Analogous to  $F_{ST}$ , the  $R_{ST}$  statistic was designed specifically for highly variable markers like microsatellites that evolve via a stepwise mutation model (SMM).  $R_{ST}$  is defined as:

$$R_{ST} = \frac{S_t - S_w}{S_t}$$

where  $S_t$  is twice the estimated variance in allele size across populations and  $S_w$  is twice the average of the estimated variance in allele size within each population. Under the SMM, mutations occur at rather high rates and involve the gain or loss of only one or a small number of repeat units for a given allele with equal probability. Populations are assumed to be of equal sample sizes and that all loci have equivalent variances, an assumption that is not often met in data sets taken from natural populations (Slatkin 1995). Both sources of bias are considered and dealt with in the RSTCALC 2.2 program by providing a further measure,  $Rho_{ST}$ , of genetic differentiation to compensate for the fact that populations are not of equal sample size nor are all loci equivalent in variance.  $Rho_{ST}$  is an unbiased estimator of Slatkin's  $R_{ST}$  where estimates are provided for both individual loci and over all loci, calculated across total populations and for all pairwise population comparisons. Statistical significance for  $Rho_{ST}$  was calculated by permutation tests with bootstrapping to provide 95% confidence intervals with 1000 iterations.

#### II.6.5.1.2.4 $(\delta\mu)^2$

The RSTCALC 2.2 program was also used to compute an additional distance measure,  $(\delta\mu)^2$  (Goldstein et al. 1995), which is specifically designed for microsatellite data and depends on time rather than population size.  $(\delta\mu)^2$  is defined as:

$$\delta\mu^2 = (\mu_A - \mu_B)^2$$

where  $\mu_A$  and  $\mu_B$  are the mean allele sizes in populations A and B respectively.  $\mu_A$  and  $\mu_B$  are calculated by first determining the average allele size at each locus in each population (Goodman 1997). The squared difference in mean allele size is then averaged over loci. If each population is at mutation-drift equilibrium, the variances within each population remain (on average) constant over time, and the linear growth in the average squared distance is a result of the squared difference between the means, then  $(\delta\mu)^2$  can be used to estimate the time since divergence of two isolated populations (Goldstein et al. 1995). As time increases,  $(\delta\mu)^2$  should increase in a linear fashion.

#### II.6.5.1.2.5 Testing for a recent bottleneck event

A population that has undergone a recent reduction in effective population size should show a reduction in the number of allele numbers and gene diversity at polymorphic loci. While gene diversity is reduced slower than the allele numbers, the observed gene diversity in a recently bottlenecked population should be higher than the expected equilibrium gene diversity which is computed from the observed number of alleles, under the assumption of a population in equilibrium (Luikart et al. 1998). All calculations were performed in the program BOTTLENECK 1.2.02 (available at: [www.montpellier.inra.fr/URLB/bottleneck/bottleneck.html](http://www.montpellier.inra.fr/URLB/bottleneck/bottleneck.html); Cornuet and Luikart 1996). Few microsatellite loci follow a strict one step SMM model, therefore we used the intermediate two-phased model (TPM) due to the proposed better fit of microsatellite data to this model (Di Rienzo et al. 1994). Given the details of our data set, a Wilcoxon test was performed due to its relatively high power (Cornuet and Luikart 1996).

### II.6.5.2 *mtDNA analysis*

The extent of genetic variation between putative populations was tested using a suite of molecular analysis techniques for the highly variable mtDNA control region. Individuals from the GOM (n=96), MED (n=19), NSEA (n=18) and the NAO (n=22) were sequenced for 399 bp of the mtDNA control region. Our sequences were aligned with previously published sequences (Lyrholm and Gyllenstein 1998) and those provided by SWFSC. The resulting haplotypes were used to provide an analysis of population structure based on maternally inherited mtDNA.

#### II.6.5.2.1 Standard diversity measures

Standard measures of diversity including haplotype frequencies, haplotype and nucleotide diversity ( $h$  and  $\pi$  respectively) (Nei 1987), mean number of pairwise differences between all haplotype pairs in the sample and the number of sequence polymorphic sites were calculated for all samples and for each putative population with ARLEQUIN 2.0 software.

Nei's (1987) measure of gene diversity ( $h$ ) is defined as the probability that two randomly chosen haplotypes are different in the sample, or in our case each putative population, and can be estimated as:

$$h = \frac{n}{n-1} \left( 1 - \sum_{i=1}^k p_i^2 \right)$$

where  $n$  is the number of gene copies in the sample,  $k$  is the number of haplotypes and  $p_i$  is the sample frequency of the  $i$ -th haplotype (Schneider et al. 2000). This measure is equivalent to the expected heterozygosity for diploid data.

Nucleotide diversity ( $\pi$ ) is equivalent to gene diversity at the nucleotide level and can be defined as the probability that two randomly chosen homologous nucleotides are different (Nei 1987). Diversity was calculated based on the Tamura and Nei (1993) model that assumes a greater rate of transitional than transversional substitutions and also allows for different rates between pyrimidine (T and C) transitions and purine (A and G) transitions. Gamma distribution correction values were set at  $\alpha = 0.47$ .

A mismatch distribution test was performed for each population using 1,000 replicates in a bootstrap approach to determine the distribution of the observed number of differences between pairs of haplotypes (Schneider et al. 2000). It then compares the observed value with the expected distribution value. When populations pass through a recent demographic expansion the distribution is usually unimodal (Slatkin and Hudson 1991; Rogers and Harpending 1992). When populations are at demographic equilibrium the distribution is usually multimodal (Slatkin and Hudson 1991; Rogers and Harpending 1992). A raggedness index ( $r$ ) of the observed distribution was computed as follows:

$$r = \sum_{i=1}^{d+1} (x_i - x_{i-1})^2$$

where  $d$  equals the greatest number of observed differences between haplotypes and the  $x$ 's are equal to the observed relative frequencies of the mismatch classes (Harpending 1994). The raggedness index takes larger values for multimodal distributions that are frequently found in stable populations than for unimodal and smoother distributions that are generally associated with expanding populations.

#### II.6.5.2.2 Selective neutrality tests

The selective neutrality of the mtDNA control region in sperm whales was assessed for each putative geographic population using Tajima's  $D$  statistic (Tajima 1989) calculated with ARLEQUIN 2.0 software. The fact that mtDNA sequences are generally short and lack recombination makes this test appropriate due to its infinite-site model without recombination basis. The  $D$  statistic's significance is tested by generating 10,000 random samples under the hypothesis of selective neutrality and population equilibrium (Tajima 1989). However, factors other than selective effects such a population expansion, bottleneck, or heterogeneity of mutation rates can also produce significant  $D$  values (Tajima 1989). Fluctuations in a population's size can result in deviation from the neutral patterns of nucleotide variation that are expected at equilibrium. If a population maintains a constant size, then variation at a neutrally evolving locus is expected to have a  $D$  value approximately equal to zero. A decrease in population size results in rare frequency mutations being lost more readily than common mutations and positive  $D$  values are expected. When

populations are under expansion, negative  $D$  values are expected as a result of a temporary excess of new mutations segregating at rare frequencies.

Fu's  $F_S$  test (Fu 1997) of selective neutrality was also calculated using ARLEQUIN 2.0 software. Fu's test evaluates the probability of observing a random neutral sample with a number of alleles similar or smaller than the observed value (Fu 1997). Although Fu's  $F_S$  test is similar to Tajima's  $D$  test in that it is based on the infinite-site model without recombination and thus ideal for the short DNA sequences we are investigating, it is more sensitive to population demographic expansion than Tajima's test, which often leads to large negative  $F_S$  values (Schneider et al. 2000).

#### **II.6.5.2.3 Divergence between populations**

The level of genetic differentiation and divergence between populations was determined using  $F_{ST}$  and  $\Phi_{ST}$  statistics, an AMOVA (Excoffier et al. 1992) and an exact test. All tests were calculated using ARLEQUIN 2.0 software. Differentiation for mtDNA loci was evaluated using  $F_{ST}$  and  $\Phi_{ST}$  statistics. An AMOVA and an exact test of heterogeneity (Raymond and Rousset 1995) were used to determine genetic differentiation within and among the four putative geographic populations.

##### **II.6.5.2.3.1 $F_{ST}$**

$F_{ST}$  was used to provide a measure of the overall haplotype frequency differentiation between the geographic populations. Calculations were performed using ARLEQUIN 2.0 software. Statistical significance was tested by 10,000 permutations of the data.

##### **II.6.5.2.3.2 AMOVA and $\Phi_{ST}$**

The partitioning of variation between geographic areas was investigated with an AMOVA to determine the degree of subdivision, if any, between regions. The AMOVA takes into account the number of mutations between haplotypes and then bases its approach on the analyses of variance of gene frequencies. The  $\Phi_{ST}$  statistic

provides a measure of differentiation for mitochondrial DNA by incorporating both haplotype frequency and genetic distance data (percent nucleotide difference) into the calculation (Excoffier et al. 1992).  $\Phi_{ST}$  values were tested for statistical significance via 10,000 permutations of the data.

#### II.6.5.2.3.3 Exact Test

An exact test of population differentiation utilizes haplotype frequency data to test the hypothesis of a random distribution of  $k$  different haplotypes among  $r$  populations (Raymond and Rousset 1995). The exact test is analogous to Fisher's exact test on a  $2 \times 2$  contingency table that has been extended to an  $r \times k$  contingency table where each row represents a population and each column an allele. The test was performed using 100,000 Markov chain steps for increased statistical significance. Probabilities of observing a table less or equally likely than the observed sample configuration under the null hypothesis of panmixia are estimated (Schneider et al. 2000). Highly significant heterogeneity was indicated by  $p$ -values less than 0.0001.

### II.7 GENERAL STATISTICS USED

Means and associated standard deviations for analysed data were generated using the program STATISTICA 5.1 (StatSoft, Inc.) and STATXACT 6.0 (Cytel Software Corp.).



### ***III. Behavioural Responses of Sperm Whales in the Northern Gulf of Mexico to Biopsy Darting for Genetic Analysis***

#### **III.1 INTRODUCTION**

Biopsy darting of free-ranging cetaceans provides researchers with a non-lethal method of obtaining small cores of skin tissue for molecular analyses. Although ethical questions will always arise with the use of any invasive technique, previous studies on numerous cetacean species have shown that reactions to biopsy darting are generally mild and short-term, and cause no long-term behavioural or wound healing complications (Best and Butterworth 1980a; Brown et al. 1991; Palsboll et al. 1991; Weinrich et al. 1991; Barrett-Lennard et al. 1996; Weller et al. 1997; Hoelzel et al. 1998b; Gauthier and Sears 1999; Hooker et al. 2001).

While DNA can be extracted from a variety of cetacean tissues, including sloughed skin, rectal cells, blood, bone, liver and skin (Amos 1997), skin is the preferred tissue by cetacean geneticists given its slow cell degradation after death, relatively high volume of DNA and relative ease of collection from free-ranging cetaceans. The collection of sloughed skin from the water column, tissue sampling of dead 'stranded' whales and biopsy sampling of free-ranging whales can all be used to obtain DNA from skin cells. Sperm whales are one of several species of cetaceans that are known to naturally slough their skin (Amos 1997). Floating patches of skin (occasionally up to one square metre in size) are often found after bouts of social or aerial activity, as well as in the fluke 'footprint' left behind after the whale arches its tail and submerges for a deep dive. Previous sperm whale genetic studies have incorporated DNA extracted from sloughed skin with reasonable success (Richard et al. 1996a, 1996b; Christal 1998; Lyrholm and Gyllenstein 1998; Bond 1999). While sloughed skin may be considered by some to be a less invasive means for gathering genetic data on whales, there are several problems that may occur with its use (including low DNA yield and DNA degradation (Bond 1999; Valsecchi et al. 1998)).

Although tissue samples collected from stranded whales allow researchers to gather essential information from deepwater species that are otherwise relatively inaccessible, it should be recognised that factors such as currents, tides or illness may

result in an unnatural location on the beach. As an alternative, biopsy sampling that utilizes a crossbow or air rifle propelled dart with corer tip to collect a small plug of tissue from the underside of the tail flukes (Figure II.2, Chapter 2) or below the dorsal hump (Figure II.3, Chapter 2) provides an extremely reliable means of obtaining a suitable sample from free-ranging cetaceans.

#### III.1.1 Behavioural Data

In order to assess a reaction to darting, pre-darting behaviour must first be described. The behavioural state (logging, milling and travel) of sperm whales prior to biopsy darting was noted based on behaviours described by Hooker et al. (2001). An important consideration in assessing how a whale reacts to the darting procedures is the extent of impact that the vessel carrying the biopsy crew may play. Sperm whales are notorious for shallow diving (called 'slip-under') if approached at too close of a range by the research vessel. As the boat slowly makes its close approach for biopsy darting, whales often become agitated and a reaction occurs. If a dorsal hump area (later referred to as the 'body') darting attempt is to be made, then the dart is released as the whale begins to arch its back just before it 'shallow dives'. This boat response could be confounded with possible responses to darting. Therefore, reactions were separated into pre-, during and post-darting time frames. That way, a whale avoiding the boat before being biopsy sampled could be distinguished from one that changes its 'normal' behaviour during or after darting occurs. Individual sperm whales are not easily identified while at the surface. As a result, a longer duration post-reaction assessment similar to what Barrett-Lennard et al. (1996) conducted for individual orca was rarely possible.

Behavioural reaction data was collected from a small boat. When possible, reactions to biopsy sampling were video taped using a digital camcorder to reconfirm the pre-, during and post-darting behaviours. For consistency, only behavioural reactions witnessed first-hand from the small boat by myself, or reactions that were documented with video and later analyzed by myself were included in these analyses. Each biopsy 'hit' was classed as follows:

1. Successful hit: where the dart made contact with the whale and a tissue sample was retained within the corer tip.

2. Unsuccessful hit: where the dart made contact, but a tissue sample was not retained within the corer tip.

Reaction levels were defined as follows (after Weinrich et al. (1991), described for humpback whales):

1. No reaction: whale continued on with the same pre-biopsy behaviour with no detectable change.
2. Low-level reaction: mild modification of behaviour (e.g. shallow dive or flinch).
3. Moderate reaction: short-term, but more forceful, modification of behaviour (e.g. tail slap, forceful upwards tail sweep, inverted arch ('banana'), acceleration and fluke-up).
4. Strong reaction: whale modified its behaviour in a succession of forceful activities or to high energy behaviour (e.g. breaches, tail slaps).

### III.2 QUESTIONS TO ADDRESS

As part of the requirements of the Marine Mammal Protection Act/Endangered Species Act (MMPA/ESA) permit #909-1465, the results presented here address the following:

1. Describes the short-term surface behavioural reactions to biopsy sampling of individuals.
2. Examines whether responses to successful sampling differ significantly between males and females.
3. Provides comparisons between the four reaction levels for an assessment of how this technique may or may not alter the behaviour of free-ranging sperm whales.

### III.3 RESULTS

A total of 43 'hits' from darts fired at northern Gulf of Mexico sperm whales were utilized in this assessment of surface behavioural reactions to biopsy sampling. Forty-two of the 43 attempts included accompanying video footage that allowed descriptions taken in the field to be reconfirmed via frame-by-frame analysis. Of the 43 hits, 34 were classed as successful (hits that retained a sample) and nine were

classified as unsuccessful (hits that made contact, but did not retain a sample) (Table III.1). The majority (91%) of successful hits were taken from the area around the dorsal hump (body). Three of the successful hits collected a sample from the tail flukes during fluke-up dives. Overall, whales reacted to 88% of all successful and unsuccessful hits. The majority of successful hits that occurred near the dorsal hump area were classed as low (48%) or moderate-level (45%) surface reactions. Similar results, 44% low-level and 56% moderate-level, were witnessed during unsuccessful hits to the body. No noticeable behavioural reaction was visible for any of the whales where samples were collected from the tail flukes during fluke-up dives. During 24 (56%) of the darting attempts, either the research vessel or an accompanying whale within the same cluster as the targeted whale was believed to instigate a pre-darting reaction from the targeted individual.

The re-sampling of individuals was not intended, but unavoidable in some cases due to difficulties in identifying previously sampled whales from others logging at the surface. Four separate whales (2 males and 2 females) were darted multiple times during the same day. During each of the initial approaches prior to darting, all four whales showed no visible reaction to the vessel. After the first successful darting attempt, two of the whales (1 male and 1 female) reacted to the approaching vessel with a shallow dive response when they were unintentionally resampled. The male's reactions were classed as moderate for both the first and second darting hits. Reactions for the female (sampled three separate times in the same day) were classed as moderate for the first darting hit and low for the second and third darting hits. The male that did not react to the approaching vessel during the second darting attempt did exhibit a moderate response to darting during the first hit and a low-level reaction to the second hit. The female that did not react to the approaching vessel during the second darting attempt showed no reaction to the first darting hit, but showed a moderate reaction to the second darting hit. However, this female was initially sampled during an ongoing tail lobbing event that may have masked the impact of the dart.

Twenty-six percent of successful hits were from males and the remaining 74% were from females (Table III.2). No significant difference in reaction levels was observed between males and females (Fisher Exact  $\chi^2 = 0.874$ ,  $p > 0.05$ ). However, sample sizes were small and may not portray an accurate representation of the population.

**Table III.1** Reaction levels for successful and unsuccessful hits to the body and flukes.

Sampling Event	Reaction Level				Total
	No	Low	Moderate	Strong	
Successful Hit – Body	2 (7%)	15 (48%)	14 (45%)	0	31
Successful Hit – Fluke	3 (100%)	0	0	0	3
Unsuccessful Hit – Body	0	4 (44%)	5 (56%)	0	9
Unsuccessful Hit – Fluke	0	0	0	0	0

**Table III.2** Reaction levels of biopsied individuals based on gender.

Gender	Reaction Level				Total
	No	Low	Moderate	Strong	
Male – Body	0	4	5	0	9
Male – Fluke	0	0	0	0	0
Female – Body	2	11	9	0	22
Female – Fluke	3	0	0	0	3

### III.4 DISCUSSION

The results presented here indicate that biopsy darting is unlikely to cause strong-level surface reactions in northern Gulf of Mexico sperm whales. Behavioural responses appeared relatively mild and short-term and consisted mainly of either a shallow dive (low-level response) or a 'banana' (inverted bow) followed by a shallow dive (moderate level response). No detectable response was seen for the three fluke sampling hits, however, given that a fluking whale is near total submergence and out of view, fluke sampling responses may not provide an accurate representation of surface behavioural reactions to darting. Although fluke sampling is far more difficult from a sampling perspective than dorsal hump area 'body' shots, the combination of biopsy sampling and photoID of the tail flukes has the advantage of securing the ID at the time of darting. This study's findings agree with those of Whitehead et al. (1990), who described mild "startle" responses by all sperm whales hit with a dart in addition to darts hitting the water near whales and close approaches by the research vessel. Whitehead et al. (1990) also noted that whales appeared to return to their normal behaviour soon after darting procedures were completed. This suggests that behavioural reactions to biopsy darting are most likely short-term. A novel result from my study is the observation that repeat biopsy events on the same individuals did not lead to increasing responses, though a larger sample size would be needed to confirm this.

## ***IV. Genetic Structure of Four Putative Geographic Sperm Whale 'Populations'***

### **IV.1 INTRODUCTION**

Geographic barriers (e.g. narrow straits) in the marine environment, or the lack thereof, may provide little influence as to how genetic structure is shaped in cetacean populations. In general, large whales are highly mobile and possess the ability to move over massive distances (see Stevick et al. 2002 for a review). However, migratory patterns may bring whales from different 'stocks' to similar breeding and feeding grounds where mixing can and does occur (Palumbi and Baker 1994; Larsen et al. 1996). The extent of social structure (e.g. matrilineal based groups) and resource specialization within a cetacean species may play a role into how one putative population varies from another (Hoelzel et al. 1998a; Hoelzel 1998b; Hoelzel et al. 1998c; Whitehead 1998). Female philopatry and male dispersal are the expected patterns of dispersion for mammalian species based on theoretical considerations (Greenwood 1980). However, there are relatively few studies that confirm this, or show clear evidence for the geographic range over which males can effect genetic dispersal. Anthropogenic factors such as harvesting may be also be responsible for the lack of genetic variation brought on by a population bottleneck, reductions in range and local extinctions for certain marine mammals species (Hoelzel et al. 2002a; Hoelzel et al. 2002b).

Sperm whales are cosmopolitan in distribution (Rice 1989), rivalled in this respect only by killer whales (*Orcinus orca*). Global post-whaling abundance estimates for sperm whales were thought to be approximately 2,000,000 (Evans 1987; Rice 1989; Berta and Sumich 1999), but Whitehead (2002) offers a recent estimate of just 360,000 whales. These whales exhibit the greatest degree of sexual dimorphism among cetaceans (Best 1979; Rice 1989). Aside from the solitary or occasional pairs of sexually and physically mature males that typically range over large distances on their own (Best 1979; Rice 1989; Whitehead 1993; Whitehead and Weilgart 2000), sperm whales are predominantly found in mixed sex social groups and units, while young males form loose aggregations called bachelor groups (Best 1979; Whitehead and Arnborn 1987; Childerhouse et al. 1995; Lyrholm and Gyllenstein 1998; Lettevall et al. 2002).

With the recent expansion of the oil and gas exploration and development industry into relatively untouched deepwater habitats occupied by a variety of cetaceans in the Gulf of Mexico, questions of population structuring and potential isolation between geographic stocks of sperm whales were addressed to provide management with a genetic assessment of structuring required to ensure anthropogenic activities do not have adverse effects on what's presumed to be a rather small number of whales. Resightings of whales throughout the year and the narrow opening through the Strait of Gibraltar may provide the Mediterranean Sea sperm whale population with a similar structuring scenario to that of the Gulf. Year-round sightings and re-sightings of individual whales over periods of days to years from numerous distribution and abundance surveys and research cruises in the northern Gulf of Mexico and the Mediterranean Sea suggest that some sperm whales exhibit a degree of philopatry to these geographic areas (Davis et al. 1998; Weller et al. 2000; Waring et al. 2001). However, both regions are relatively unknown with regards to stock identity and geographic separation.

#### **IV.1.1 Sperm Whale Genetic Structure (Previous Findings)**

Various types of data (including dialects, genetics, mark-recapture data, morphology, parasitism and predation (Best 1979; Whitehead 1987; Whitehead and Arnborn 1987; Arnborn and Whitehead 1989; Rice 1989; Whitehead and Kahn 1992; Dufault and Whitehead 1995; Lyrholm and Gyllentsen 1998; Whitehead et al 1998; Lyrholm et al. 1999) suggest philopatry among female sperm whales, while adult males are known to be capable of ranging over vast distances (Best 1979; Rice 1989; Whitehead and Weilgart 2000). Recent population structure analysis based on both matrilineal and bi-parental genetic markers, involving mtDNA and microsatellites analyses respectively, are consistent with the expectation of greater female than male philopatry in this species (Lyrholm et al. 1996, 1999; Lyrholm and Gyllensten 1998; Bond 1999). These studies have indicated substantially low levels of nucleotide variation on a global scale and the presence of significant levels of kinship between some group members that may be the result of matrilineal structuring at the unit or group level (Lyrholm et al. 1996, 1999; Richard et al. 1996a; Christal 1998; Lyrholm and Gyllensten 1998; Whitehead et al. 1998; Bond 1999). Lyrholm et al. (1996) proposed a historical bottleneck to explain the low levels of mtDNA diversity (based



on DNA sequence data of 320bp from the control region). Estimated time since a possible bottleneck event happened for sperm whales ranged from approximately 6,000 to 25,000 years ago; which coincides with the end of the Pleistocene glaciation era (Lyrholm et al. 1996). Although the level of mtDNA genetic structure between global populations was low, there were statistically significant patterns of differentiation between oceans suggesting philopatry within females groups to particular regions (Lyrholm and Gyllenstein 1998). By comparison, studies examining nuclear DNA heterogeneity in polymorphic microsatellite allele frequencies revealed either no significant (Lyrholm et al. 1999) or low, but significant (Bond 1999) degrees of population structuring between oceans. In addition, the same studies failed to detect any differentiation for smaller scale geographic comparisons within either the North Pacific or North Atlantic Oceans. Private alleles to particular geographic areas within the North Pacific and North Atlantic Oceans were rare. Several cetacean studies have shown that as geographic distances between populations increase, population differentiation between the two populations increases as well (Berube et al. 1998; Pichler et al. 1998; Valsecchi et al. 1997). When comparing multiple geographic areas, an increase in genetic differentiation corresponding to an increase in geographic distance was not supported by Bond's (1999) results. However, sample sizes from several locations were small and may provide misleading results. The combination of behavioural and genetic studies (using bi-parental nuclear DNA (i.e. microsatellite markers) in comparison with the matrilineally inherited mtDNA) provide a strong indication that extensive movement by sexually mature males among female structured populations may be occurring (Bond 1999; Lyrholm et al. 1999).

#### **IV.2 QUESTIONS TO ADDRESS**

To examine the level of genetic variation and differentiation between the Gulf of Mexico (GOM), Mediterranean Sea (MED), North Sea (NSEA) and the North Atlantic Ocean (NAO) 'geographic' populations, we analyzed the following:

1. Assess the degree of sequence variability in the mtDNA control region across three previously unstudied populations and the level of variation existing between the GOM, MED, NSEA and the larger (partially studied) NAO population. Small-scale population structure is unexpected for this species

due to their extensive ranging behaviour, and especially with respect to the movement of males. However, there are some data to suggest that within the GOM and the MED regions there may be groups of whales that remain in or return to a local geographic region, possibly to exploit an abundant and seasonally consistent resource. Given its maternal mode of inheritance, mtDNA variability between populations may provide an assessment of population structuring with respect to the movement of females.

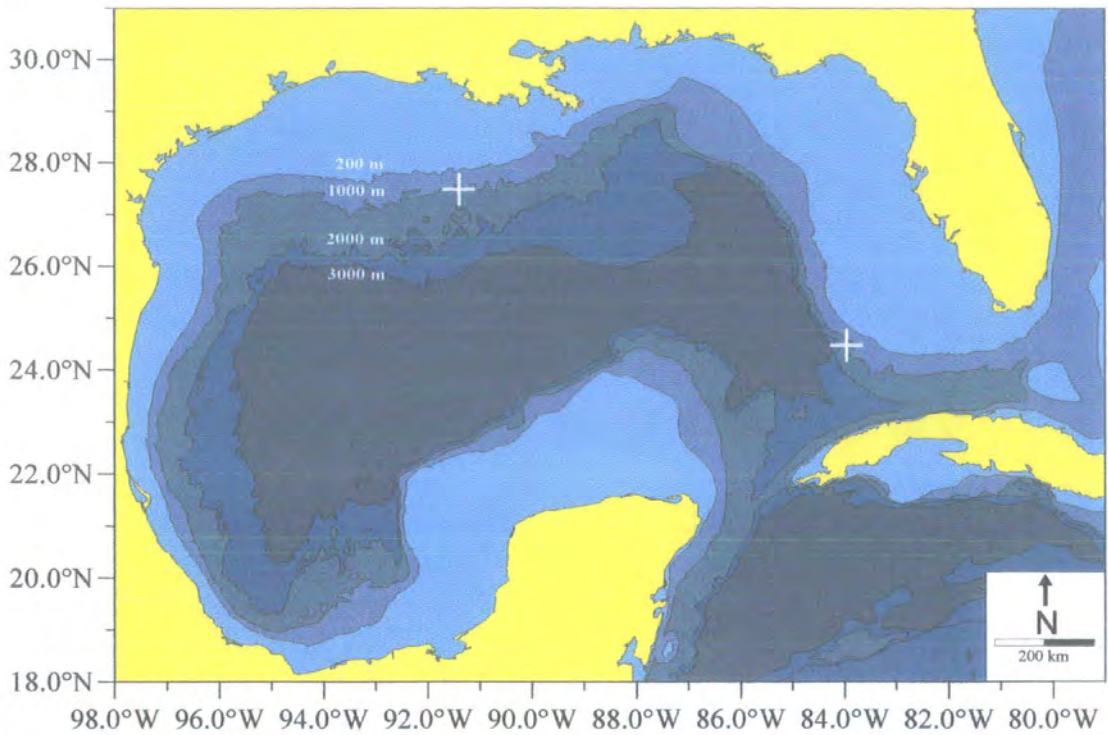
2. Assess the extent of allelic variation between putative populations across multiple nuclear microsatellite loci. While there is evidence of geographic structuring between oceans with respect to the movement of females (using mtDNA markers), there is little evidence for structuring at nuclear DNA markers. My study uses the same genetic markers across geographic regions and therefore permits an assessment of the extent and range of gene flow between whales in the GOM, MED and the NSEA (published microsatellite data was unavailable for comparisons with the NAO population). This analysis using bi-parentally inherited nuclear markers, in addition to maternally inherited mtDNA markers, will allow an assessment of the movement patterns of both males and females.

### **IV.3 RESULTS**

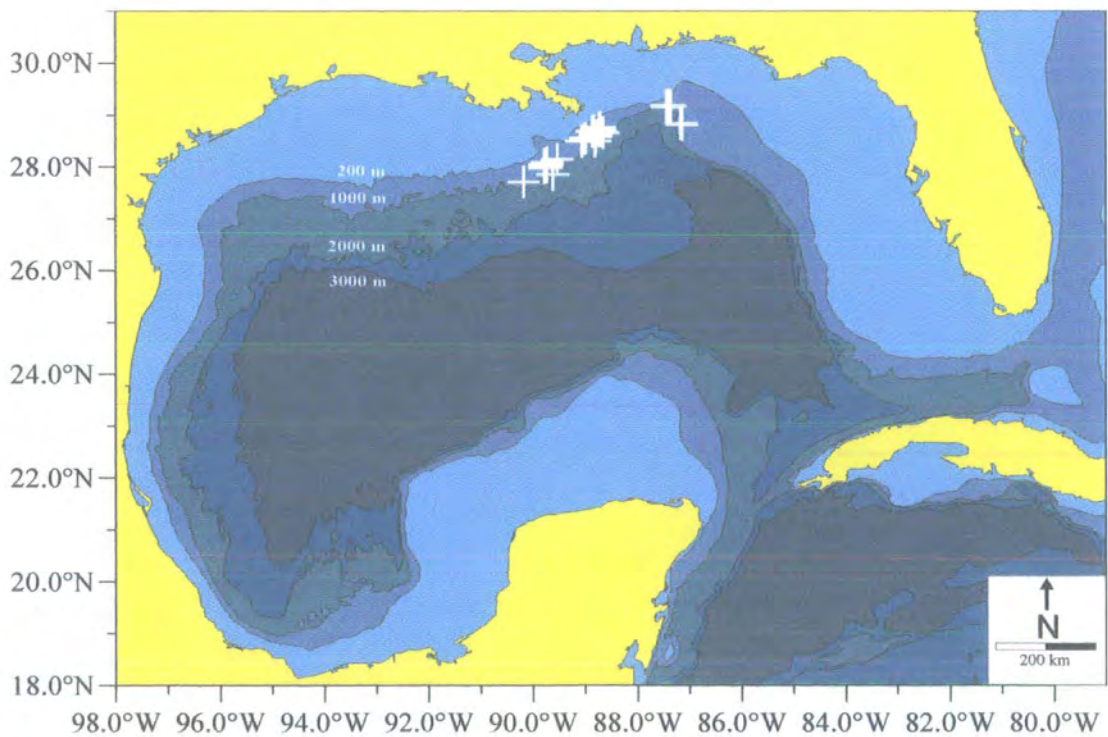
#### **IV.3.1 Sampling Locations**

A total of 156 tissue samples comprised of biopsies ( $N = 118$ ) and sloughed skin ( $N = 38$ ) were collected from free-ranging sperm whales located in the northern GOM during six research cruises (Figures IV.1 – IV.5). All free-ranging whale samples collected during cruises in the northern GOM from May 2000 to September 2002 are shown in Figure IV.6. A further four samples were collected from stranded whales in the GOM (Texas:  $N = 2$ , Louisiana:  $N = 1$ , Florida:  $N = 1$ ) (Figure IV.6).

Fifty samples comprised of sloughed skin ( $N = 46$ ) and biopsies ( $N = 4$ ) were collected from free-ranging whales in the MED during 1999 to 2002 (Figure IV.7). An additional three samples were collected from stranded whales in the MED (Zakynthos Island:  $N = 1$ , Pylos Island:  $N = 1$ , Andros Island:  $N = 1$ ) (Figure IV.7).

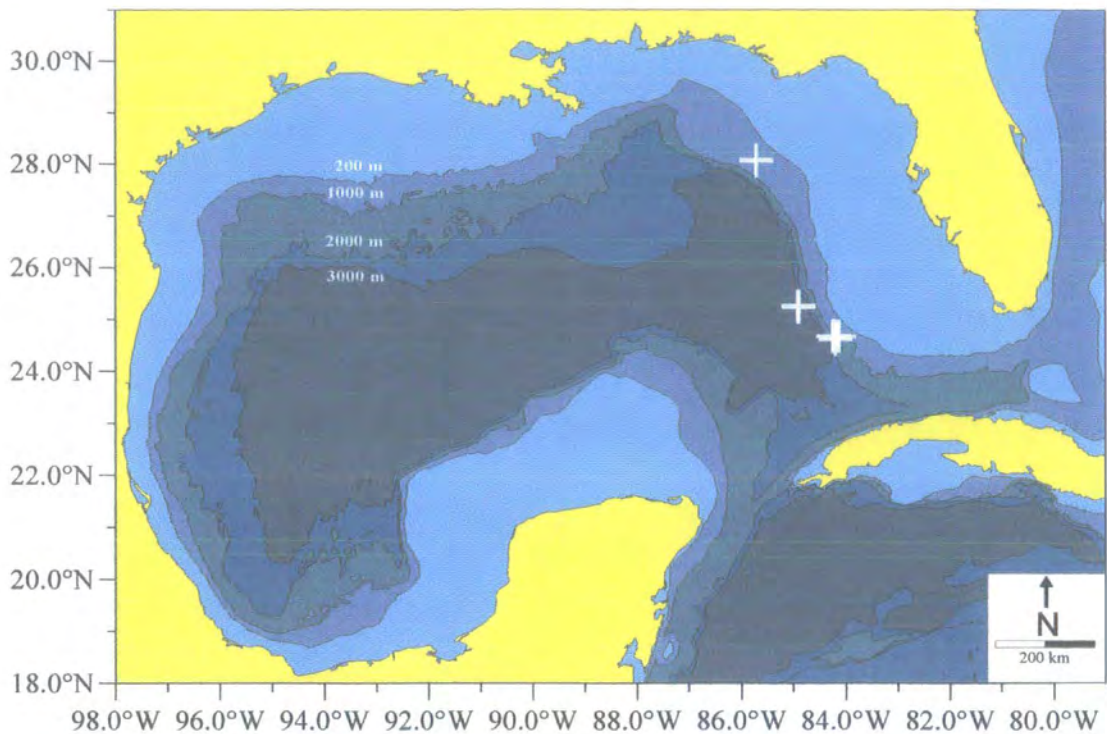


**Figure IV.1** GOM biopsy sampling locations during the May 2000 NMFS marine mammal survey cruise are depicted as white crosses. Depth contours of 200m, 1000m, 2000m and 3000m are illustrated in shades of blue.

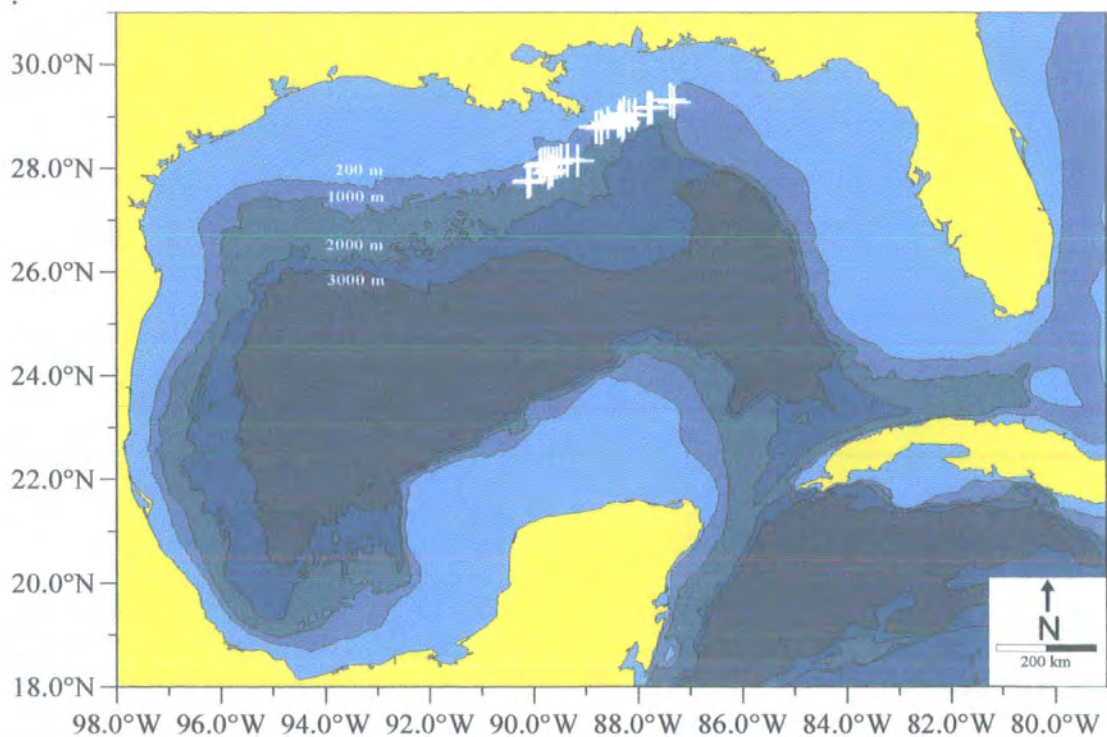


**Figure IV.2** GOM biopsy and sloughed skin sampling locations during the SW2K July – August 2000 SWAMP cruise are depicted as white crosses. Depth contours of 200m, 1000m, 2000m and 3000m are illustrated in shades of blue.

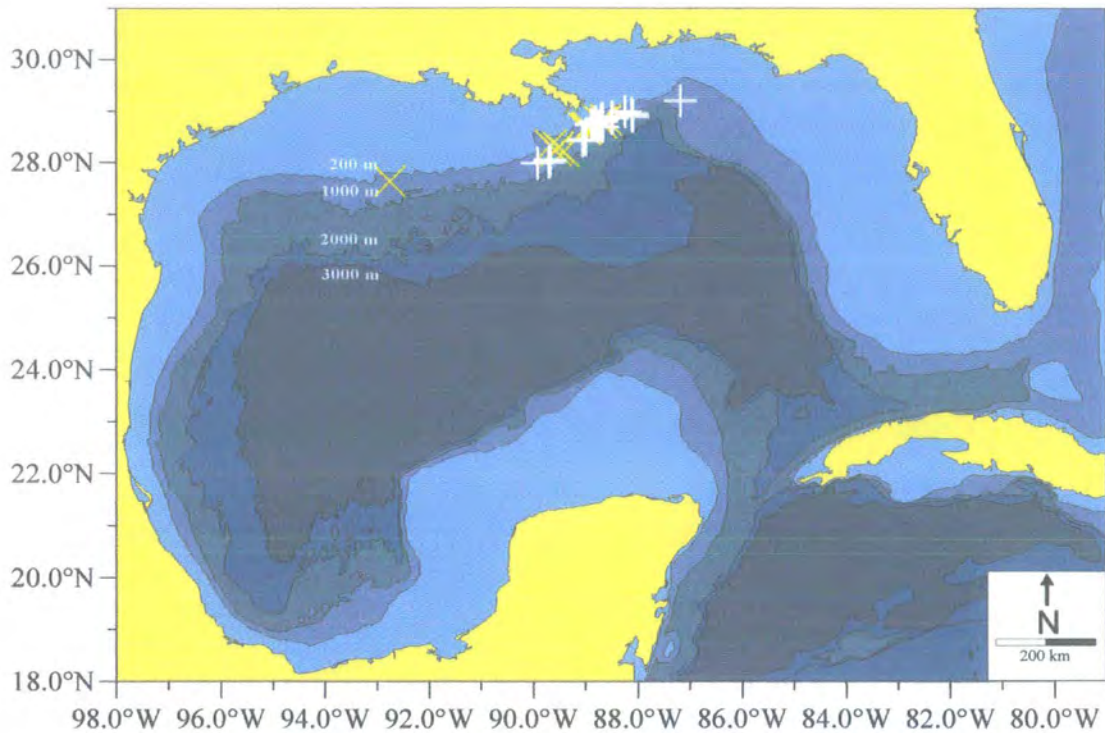




**Figure IV.3** GOM biopsy and sloughed skin sampling locations during the SW2K1 March – April 2001 SWAMP spring cruise are depict as white crosses. Depth contours of 200m, 1000m, 2000m and 3000m are illustrated in shades of blue.

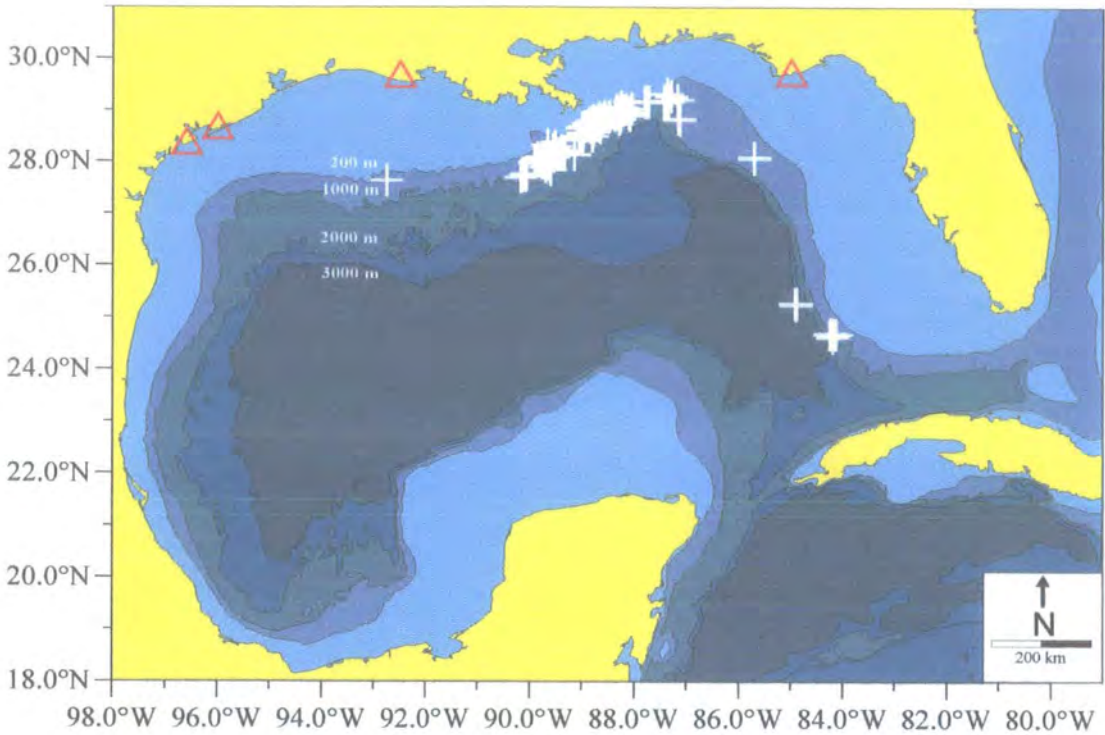


**Figure IV.4** GOM biopsy and sloughed skin sampling locations during the SW2K1 July to August 2001 SWAMP summer cruise are depicted as white crosses. Depth contours of 200m, 1000m, 2000m and 3000m are illustrated in shades of blue.

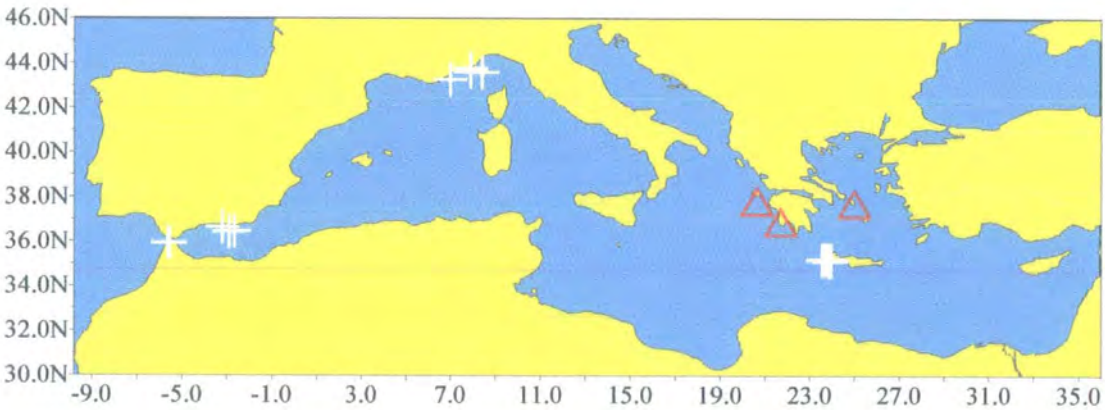


**Figure IV.5** Biopsy and sloughed skin sampling locations during the June to July and August to September 2002 SWSS cruises. White crosses (+) represent whale samples collected during the June to July satellite-tagging leg of the cruise, while samples denoted with a yellow 'X' are from samples collected during the August to September digital acoustic recording tag (DTAG) leg. Depth contours of 200m, 1000m, 2000m and 3000m are illustrated in shades of blue.



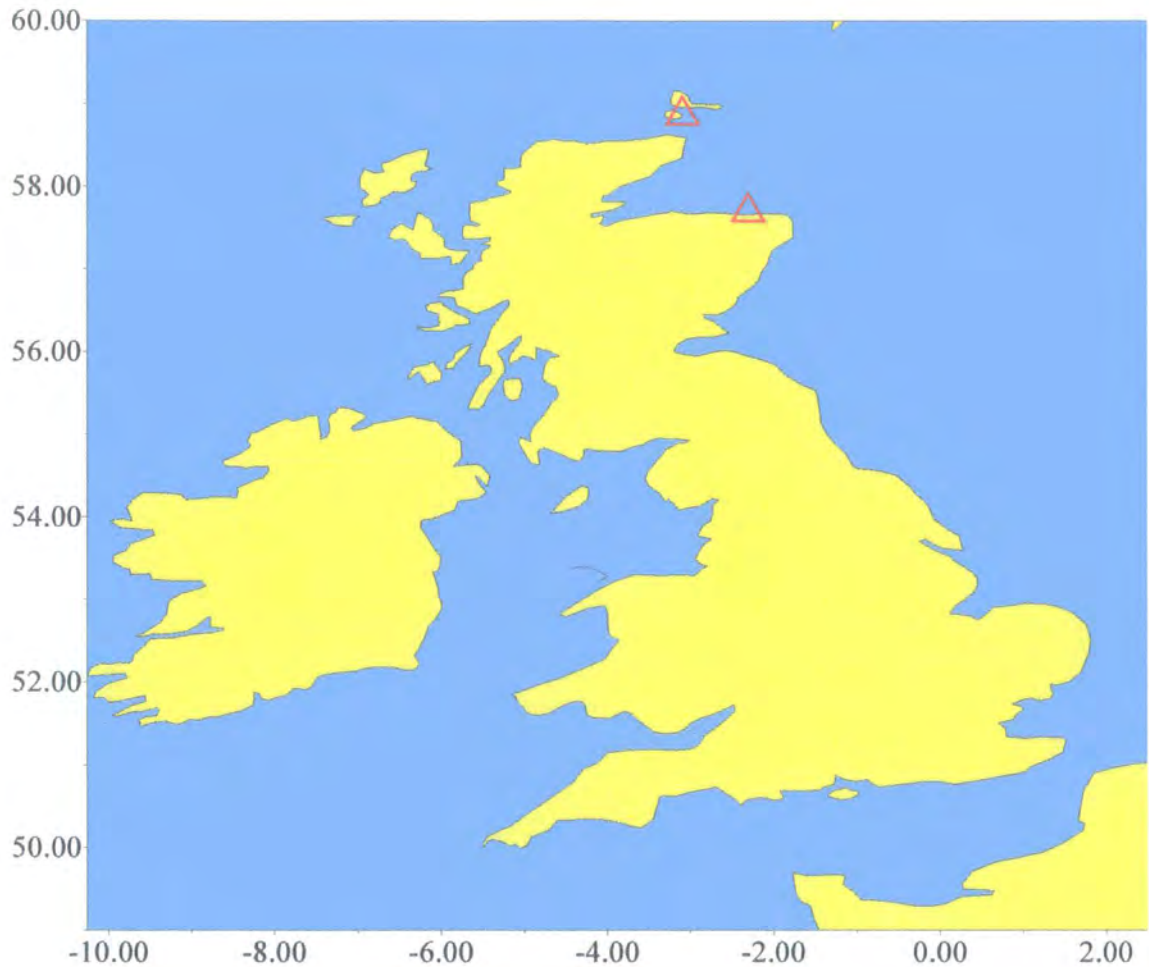


**Figure IV.6** Total free-ranging whale samples collected during all cruises in the northern GOM from July 2000 to September 2002. Red triangles represent the 4 stranded whales used in population comparisons. Depth contours of 200m, 1000m, 2000m and 3000m are illustrated in shades of blue.



**Figure IV.7** Locations of biopsy and sloughed skin samples collected from free ranging whales in the MED are depicted with a white cross. Stranded whale sample locations collected in the MED are depicted with a red triangle. Note: the water is illustrated as blue, but depth contour lines are not provided for this map.

Twenty samples collected from the NSEA were primarily taken from two stranding events along the Orkney (N = 11) and Grampian (N = 6) coasts in 1994 and 1996 respectively (Figure IV.8).



**Figure IV.8** Approximate locations of skin samples collected from two groups of stranded whales in the NSEA are denoted with red triangles. Note: the water is illustrated as blue, but depth contour lines are not provided for this map.

The NAO sample set ( $N = 86$ ) combined my 22 stranded whale samples from the NSEA ( $N = 18$ ), Caribbean Sea ( $N = 3$ ) and Florida ( $N = 1$ ) with those of Sarah Mesnick's (SWFSC) western North Atlantic set ( $N = 17$ ) that included biopsy samples in the NAO ( $N = 4$ ) and stranding events in the Bahamas ( $N = 7$ ), North Carolina ( $N = 2$ ) and Florida ( $N = 4$ ). Forty-seven samples from Lyrholm and Gyllensten's (1998) dataset were also incorporated and included samples from the Azores ( $N = 13$ ), Denmark ( $N = 15$ ), Norway ( $N = 8$ ), Iceland ( $N = 8$ ), Sweden ( $N = 1$ ), Florida ( $N = 1$ ) and the Dominican Republic ( $N = 1$ ). Precise GPS locations for those samples were unavailable. Stranded whale samples were included in both the 'all' and '*restricted*' datasets for population comparisons barring high levels of relatedness among free-ranging whales.

### IV.3.2 Identity Check

#### IV.3.2.1 *Gulf of Mexico (GOM)*

Of the 156 tissue samples that were collected during the 2000 – 2002 GOM fieldwork seasons, results from sorting individual genotypes by eye and using the CERVUS 2.0 program for confirmation indicated 56 samples were genetic duplicates of previously sampled whales. One extremely minute sample, collected incidentally from the satellite-tag deployment push rod, resulted in very poor PCR amplification for microsatellites (although mtDNA results were adequate) and was removed from population structuring due to our inability to determine whether it was a duplicate of another sample. However, this sample's mtDNA haplotype was included in a portion of the satellite tagged whale social structure analyses described in Chapter 5. By adding the four GOM strandings, our final GOM sperm whales sample set to be incorporated into the examination of relatedness and population structure was set at 103 individuals. The extent of duplication was primarily a result of the difficulty in determining individual whale identities while they were 'logging' (lying nearly motionless at the surface) due to their rather monomorphic appearance. The collection of multiple pieces of sloughed skin from lone whales, social groups or whales that had been previously biopsied was an additional factor that lead to the high number of duplicate samples. By examining the duplicate sample data we have discovered 30 whales that were sampled more than once over periods ranging from the same day to multiple years (Appendix I). The ranges of distances between resampling events were 0.00 to 16.68 km on the same day, 10.24 km to 87.03 km



between multiple days and 16.21 to 109.03 km between years (Appendix I). This data supports prior re-sighting evidence that suggests some degree of site-fidelity to the Mississippi River Canyon area may be exhibited by individual sperm whales (Weller et al. 2000).

#### **IV.3.2.2 Mediterranean Sea (MED)**

Of the 50 whales that were sampled during the 1999 – 2001 Mediterranean fieldwork seasons, results indicated that 21 samples were genetic duplicates of previously sampled whales. After initial extraction and amplification procedures, nine of the remaining 29 samples were excluded altogether due to difficulties with PCR amplification for the degraded DNA that is often associated with sloughed skin tissue. With the addition of three MED strandings, the total number of MED sperm whales incorporated into the examination of relatedness and population structure was set at 23 individuals. In this case, the majority of sample duplication was the result of researchers unintentionally collecting multiple samples of sloughed skin from the same individual while sampling groups socializing at the surface.

#### **IV.3.3 Probability of Identity**

Assuming random selection of individuals and Hardy-Weinberg Equilibrium among the populations involved, the probability of identity for each locus for the 'all' and 'restricted' datasets are shown in Table IV.1. The combination of Whitehead's (2002) global estimate of 360,000 sperm whales and the extremely low probability of identity values obtained by comparing samples across sixteen microsatellites suggests that finding a match among genotypes using all loci (ex. MED 'restricted' dataset =  $4.74 \times 10^{-14}$ ) is negligible and provides an exceptionally high level of assurance that utilization of this type of genetic typing technique can accurately identify individual sperm whales based on their genotypes. Rather than type each sample at all sixteen loci, a more cost-effective measure was to compare only the six least variable loci (EV1, EV104, D08, D22, GATA28 and GATA417). When implementing this measure for the GOM 'all' dataset, we still obtain a low probability of identity of  $7.57 \times 10^{-05}$ . As a result, the majority of samples were compared at between six and ten loci to eliminate duplicates.

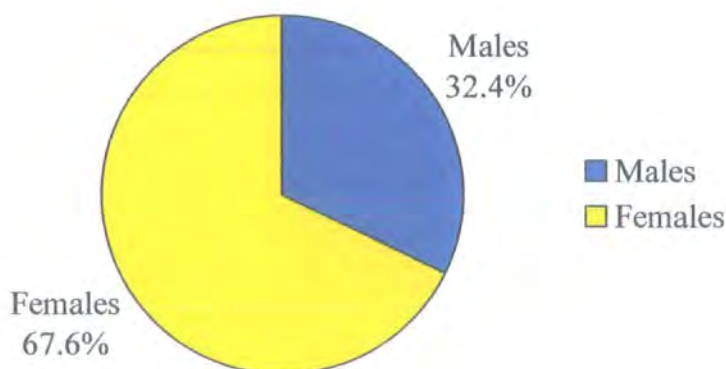
**Table IV.1** Probability of identity values for each population across 16 microsatellite loci. Calculations are based on Paetkau and Strobeck (1994).

Locus	<u>Gulf of Mexico</u>		<u>Mediterranean Sea</u>		<u>North Sea</u>	
	All	<i>Restricted</i>	All	<i>Restricted</i>	All	<i>Restricted</i>
EV1	0.219	0.143	0.579	0.532	0.214	0.196
EV5	0.131	0.082	0.086	0.067	0.114	0.112
EV37	0.016	0.016	0.031	0.075	0.024	0.022
EV94	0.063	0.040	0.052	0.063	0.052	0.043
EV104	0.128	0.132	0.180	0.191	0.160	0.168
SW10	0.031	0.031	0.040	0.054	0.033	0.032
SW13	0.063	0.062	0.228	0.293	0.066	0.060
SW19	0.017	0.016	0.050	0.095	0.021	0.021
TEXVET5	0.051	0.048	0.060	0.075	0.053	0.054
D08	0.195	0.237	0.476	1.000	0.255	0.266
D22	0.225	0.278	0.198	0.239	0.281	0.307
FCB1	0.052	0.060	0.046	0.043	0.031	0.032
FCB14	0.053	0.067	0.053	0.074	0.059	0.062
FCB17	0.013	0.014	0.091	0.118	0.018	0.017
GATA28	0.213	0.216	0.253	0.298	0.244	0.237
GATA417	0.289	0.323	0.469	0.489	0.248	0.230
Totals	$6.07 \times 10^{-19}$	$3.78 \times 10^{-19}$	$9.81 \times 10^{-16}$	$4.74 \times 10^{-14}$	$1.69 \times 10^{-18}$	$1.14 \times 10^{-18}$

### IV.3.4 Gender Determination

#### IV.3.4.1 Gulf of Mexico

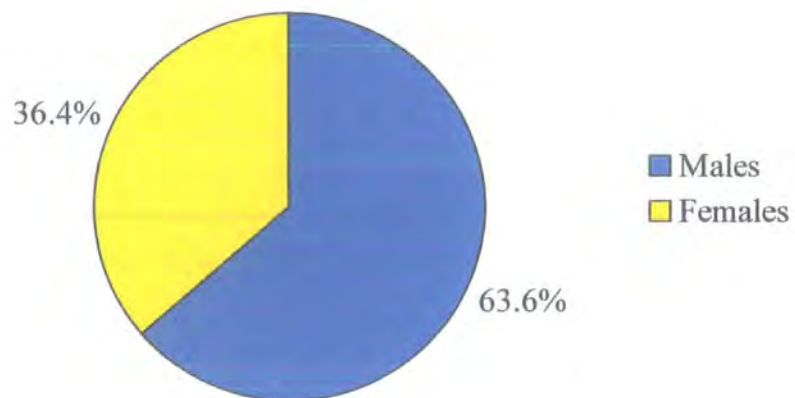
For each of the 103 unique GOM individuals, gender was determined for 102 (the missing individual failed to provide readable results due to a poor quality/quantity sample) whales using the ZFX/ZFY technique described by Berube and Palsboll (1996). Male and female strandings with known gender from the GOM and NSEA were included as a means of confirmation for PCR amplifications and yielded expected results. In the GOM, the sex ratio of females to males was 2.08:1 (0.676:0.324), which is significantly different than an expected ratio of 1:1 ( $\chi^2=12.71$ ,  $p<0.001$ ) (Figure IV.9). This is not unexpected though given what appears to be a preference for lower-latitude waters by female mixed groups (Best 1979; Rice 1989). None of the males that were sampled appeared to be both physically and sexually mature based on very rough length estimates compared to the RHIB and morphological characteristics (e.g. pronounced heads: heads that appear swollen with a distinct ridge behind the base of the skull). However, several of these 'young' males may be either in or nearing sexual maturity based on these same rough size estimates and compared to Best's (1979) sexual maturity estimates.



**Figure IV.9** Gender composition of whales sampled in the northern GOM during 2000-2002.

#### IV.3.4.2 Mediterranean Sea

Gender results were obtained from 22 of the 23 (the missing individual failed to provide readable results due to a poor quality/quantity sample) whales tested. Male and female strandings with known gender from the GOM, MED and NSEA were included as controls for PCR amplifications and yielded expected results. The MED's sex ratio of females to males was 0.571:1 (0.364:0.636), which is not significantly different than an expected ratio of 1:1 ( $\chi^2=1.64$ ,  $p>0.05$ ) (Figure IV.10). However, to say that males outnumber females in the MED may be somewhat misleading though due to the sampling methods, time frame involved and sample sizes obtained. For instance, the majority of groups cited off the island of Crete appear to fit the 'mixed' group structure scenario with numerous females and young males seen socializing at times, while whales that frequent the Strait of Gibraltar and Ligurian Sea areas appear to be young males in either smaller groups, pairs or alone. Whether these young males remain in the MED or move towards more polar latitudes remains a focus of the North Atlantic and Mediterranean Sperm Whale Catalogue (NAMSC) project. The NAMSC project is a collaborative ongoing photo-ID research effort that compares identifying photos with multiple geographic areas.



**Figure IV.10** Gender composition of whales sampled in the MED during 1999-2001.

#### IV.3.4.3 North Sea

Twenty samples collected from the NSEA were from immature males ranging in size from 12 – 15 metres in length (Mean = 12.96 m, std. dev. = 0.743). This preference for higher latitudes among young males and bachelor groups is not uncommon for sperm whales (Best 1979; Rice 1989; Childerhouse et al. 1995; Lettevall et al. 2002).

#### IV.3.5 Sample Size Reductions ('All' and 'Restricted' Sample Sets)

##### IV.3.5.1 Population comparisons – sample numbers and parameters

Previous studies suggest that high levels of relatedness found within sperm whale groups could potentially bias statistical testing when examining geographic population structuring (Richard et al. 1996a; Lyrholm et al. 1999; Bond 1999). As a result, population comparisons using mtDNA and microsatellite DNA (msatDNA) loci were performed using two datasets – 'all' and 'restricted'. Two methods were implemented to eliminate close kin from the *restricted* population structure estimates. The first used a combination of Kinbegone (SWFSC) and Relatedness (Goodnight Software) software to eliminate highly related whales. The second relied on the inclusion of only one individual sampled within a group (this pertained to Lyrholm and Gyllensten's (1998) published material and SWFSC's western NAO sequences only). Due to their semi-restricted movement patterns (Best 1979; Rice 1989; Childerhouse et al. 1995; Reeves and Whitehead 1997; Lettevall et al. 2002), young males (based on visual and preliminary photogrammetry size range estimates (P. Miller, pers. comm.; C. Cates, pers. comm)) were included in the 'restricted' set analyses, barring high degrees of relatedness to other whales in the population. Males larger than 11 meters were not excluded from the Lyrholm and Gyllensten (1998) published data set (see below) as it was assumed they had already dispersed from their natal group at the time of sampling.

The 'all' dataset included all sampled individuals within each of three geographic areas (mtDNA: GOM: N = 96, MED: N = 19, NSEA: N = 18; msatDNA: GOM: N = 83, MED: N = 22, NSEA: N = 20). The 'restricted' dataset consisted of individuals that were 'pruned' to eliminate close kin (mtDNA: GOM: N = 40, MED: N = 7, NSEA: N = 16; msatDNA: GOM: N = 40, MED: N = 8, NSEA: N = 18). Sample sizes differed from the original collected numbers due to failure of poor quality and quantity samples to be sequenced for the control region or failure for the

majority of the 16 microsatellites to amplify correctly. In some cases, mtDNA sequence numbers differed from msatDNA numbers within a population (e.g. mtDNA: GOM:  $N = 96$ , msatDNA: GOM:  $N = 83$ ) due to a failure in sequencing or the failure of the majority of the 16 microsatellites to amplify correctly for a given sample. An additional analysis for mtDNA sequence results was set up that compared the GOM and MED 'all' and '*restricted*' populations against an overall NAO population. The NAO population was a compilation of data from published studies including samples distributed throughout the western and eastern NAO and the NSEA. The NAO incorporated my 'all' and '*restricted*' sequences from the NSEA ( $N = 18$ ,  $N = 16$ ), Caribbean Sea ( $N = 3$ ,  $N = 3$ ), and the western NAO ( $N = 1$ ,  $N = 1$ ) with those of Sarah Mesnick's (SWFSC) western North Atlantic sequences ( $N = 17$ ,  $N = 5$ ) and Lyrholm and Gyllensten's (1998) published NAO sequences ( $N = 47$ ,  $N = 42$ ). NAO sequences from SWFSC were taken from biopsy samples in the North Atlantic ( $N = 4$ ,  $N = 1$ ), and stranding events in the Bahamas ( $N = 7$ ,  $N = 1$ ), North Carolina ( $N = 2$ ,  $N = 2$ ), and Florida ( $N = 4$ ,  $N = 1$ ). NAO published sequences from Lyrholm and Gyllensten (1998) were incorporated into the population structure analyses from free-ranging and stranded whales located in the following areas: North Atlantic (NAO)  $N = 47$ : Azores  $N = 13$ , Denmark  $N = 15$ , Norway  $N = 8$ , Iceland  $N = 8$ , Sweden  $N = 1$ , Florida  $N = 1$  and the Dominican Republic  $N = 1$  (restricted numbers by specific region were not available). Unfortunately, Lyrholm and Gyllensten's (1998) published haplotypes by region were unobtainable at this time so the NAO can not be split into the western and eastern NAO for a more detailed comparison of geographic areas. The total number of samples used to represent the 'all' and '*restricted*' NAO was 86 and 67 respectively.

While a sufficient number of samples remained for the GOM, NSEA and NAO '*restricted*' datasets, the reduction of possible relatives within the putative MED population significantly reduced the number of individuals used for mtDNA analyses from 19 to 7 and for microsatellite analyses from 22 to 8. Although the MED '*restricted*' data set was included in population comparisons, caution should be taken when interpreting results obtained from small sample sizes.

IV.3.6 Mitochondrial DNA

IV.3.6.1 Genetic diversity estimates

Sequences from this study for 201 individual sperm whales from the GOM, MED, NSEA and NAO were compared at the 399bp segment from the 5’ control region with twenty-three sperm whale haplotypes (organized as letters A through W and Lyr. 4) provided by Sarah Mesnick (SWFSC) using the program Mega 2.0 (<http://www.megasoftware.net/>) to determine unique haplotypes within the three geographic regions. The SWFSC data include Lyrholm and Gyllensten’s (1998) forty-seven previously sequenced samples and resulting haplotypes collected in areas throughout the NAO as well as all other haplotypes discovered to date that occur for sperm whales on a global scale.

For my study, six (1.5%) polymorphic nucleotide sites defining a total of only seven unique lineages were found between the GOM, MED, NSEA and NAO (Table IV.2). All nucleotide substitutions between haplotypes were transitions, two of which were pyrimidine and four were purine transitions, therefore no transition/transversion weighting was applied in the analyses.

**Table IV.2** Haplotypes with corresponding variable sites for 399 base pairs of the mtDNA control region for sperm whales distributed throughout the GOM, MED, NSEA and NAO. Dots indicate nucleotide equivalence with the reference sequence (HapA) above.

Haplotype	Variable Sites					
	6	1	2	2	2	3
	2	2	0	7	8	1
		1	7	2	8	9
Hap A	C	C	A	A	A	G
Hap B	T	.	.	.	.	.
Hap C	T	.	.	.	G	.
Lyr4	.	T	.	.	.	.
Hap N	T	.	.	.	.	A
Hap X	T	.	G	.	G	.
Hap Y	T	.	G	G	G	.

Shared haplotypes, distribution of haplotypes and haplotype frequencies are provided in Table IV.3. On a global scale, the three most common haplotypes were ‘A’, ‘B’, and ‘C’ (Lyrholm and Gyllensten 1998). While these three haplotypes clearly dominated the NAO (Lyrholm and Gyllensten (1998)), two haplotypes (‘X’

Table IV.3 Haplotype frequencies for four geographic putative populations. The left number indicates the 'all' data set and the right italicized number indicates the '*restricted*' subset.

Haplotype	Gulf of Mexico	Mediterranean Sea	North Sea	*North Atlantic Ocean
A	0.021 / 0.000	0.000 / 0.000	0.444 / 0.438	0.395 / 0.388
B	0.219 / 0.225	0.000 / 0.000	0.111 / 0.125	0.151 / 0.194
C	0.094 / 0.100	1.000 / 1.000	0.444 / 0.438	0.419 / 0.373
Lyr4	0.000 / 0.000	0.000 / 0.000	0.000 / 0.000	0.012 / 0.015
N	0.000 / 0.000	0.000 / 0.000	0.000 / 0.000	0.023 / 0.030
X	0.583 / 0.575	0.000 / 0.000	0.000 / 0.000	0.000 / 0.000
Y	0.083 / 0.100	0.000 / 0.000	0.000 / 0.000	0.000 / 0.000
Total	96 / 40	19 / 7	18/16	86 / 67

\*Includes published haplotypes from Lyrholm and Gyllensten (1998), sequences from SWFSC and the NSEA sample set.

and 'Y') were unique to the GOM with 'X' being the most common (58.3%). A total of five haplotypes ('A', 'B', 'C', 'X' and 'Y') occurred in the GOM, all samples collected from the MED were represented by a single haplotype ('C'), three haplotypes ('A', 'B' and 'C') occurred in the NSEA samples and five haplotypes ('A', 'B', 'C', 'Lyr4' and 'N') were present in the NAO data. Although 'A' and 'B' do occur in the GOM, NSEA and the NAO, only the 'C' haplotype was distributed across all geographic regions.

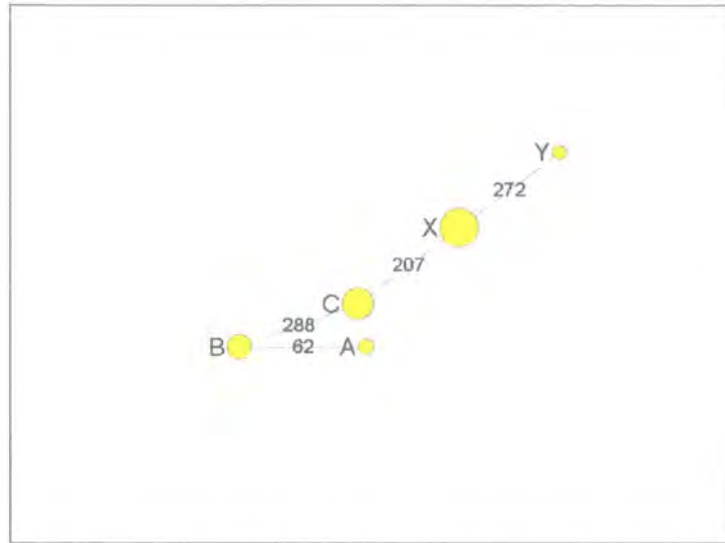
Gene (or haplotype) diversity ( $h$ ), nucleotide diversity ( $\pi$ ) and the mean number of pairwise differences for each individual population were unusually low across each of the four 'all' and '*restricted*' populations datasets (Table IV.4). Very similar results were reported by Lyrholm et al. (1996) and Lyrholm and Gyllensten (1998). The GOM, NSEA and the NAO populations all possessed similar low levels of gene diversity. Nucleotide diversity was also extremely low and constant across the GOM, NSEA and NAO populations ( $0.3\% \pm 0.2$ ). A minimum spanning network (MSN) showed no real separation of haplotypes into distinct clusters, which was as expected given the extremely low variation between haplotypes (Figure IV.11).



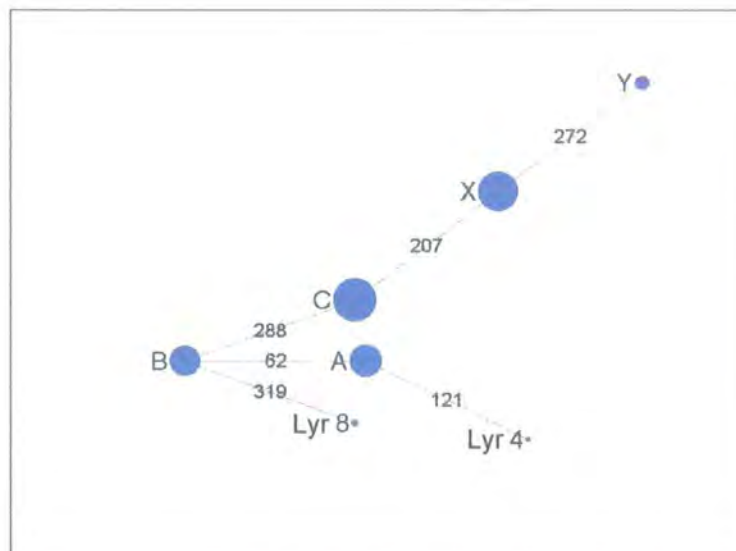
**Table IV.4** Haplotype diversity ( $h$ ), nucleotide diversity ( $\pi$ )  $\pm$  standard deviation and the mean number of pairwise differences  $\pm$  standard deviation within four geographic populations. *Restricted* dataset numbers are in italics.

Location	Dataset	$n$	Haplotype Diversity ( $h$ )	Nucleotide Diversity ( $\pi$ )	Mean Number of Pairwise Differences
GOM	all	96	0.602	$0.003 \pm 0.002$	$1.056 \pm 0.706$
	<i>restricted</i>	<i>40</i>	<i>0.614</i>	<i>0.003 <math>\pm</math> 0.002</i>	<i>1.035 <math>\pm</math> 0.705</i>
MED	all	19	0.000	$0.000 \pm 0.000$	$0.000 \pm 0.000$
	<i>restricted</i>	<i>7</i>	<i>0.000</i>	<i>0.000 <math>\pm</math> 0.000</i>	<i>0.000 <math>\pm</math> 0.000</i>
NSEA	all	18	0.628	$0.003 \pm 0.002$	$1.066 \pm 0.739$
	<i>restricted</i>	<i>16</i>	<i>0.642</i>	<i>0.003 <math>\pm</math> 0.002</i>	<i>1.071 <math>\pm</math> 0.745</i>
NAO	all	86	0.653	$0.003 \pm 0.002$	$1.072 \pm 0.715$
	<i>restricted</i>	<i>67</i>	<i>0.682</i>	<i>0.003 <math>\pm</math> 0.002</i>	<i>1.075 <math>\pm</math> 0.718</i>
Total	all	201	0.762	$0.003 \pm 0.002$	$1.349 \pm 0.842$
	<i>restricted</i>	<i>130</i>	<i>0.776</i>	<i>0.003 <math>\pm</math> 0.002</i>	<i>1.349 <math>\pm</math> 0.842</i>

The results of Tajima's  $D$  for each 'all' and 'restricted' population (excluding the MED due to total lack of polymorphism) were non-significant ( $p > 0.05$ ). Fu's  $F_S$  test confirmed these results ( $p > 0.10$  for each population). Although the resolution was low due to the small number of haplotypes present in the sample set, the mismatch distribution tests performed in ARLEQUIN 2.0 showed no significant deviation from the unimodal model that suggests expansion.



a) All haplotypes obtained from the GOM, MED and NSEA geographic areas.



b) All haplotypes obtained from the GOM, MED and NAO (includes the NSEA) geographic areas.

**Figure IV.11** Minimum spanning network (MSN) that depicts the relationships among mtDNA d-loop haplotypes for a) the GOM-MED-NSEA 'all' dataset and b) the GOM-MED-NAO 'all' dataset. Circle diameters are roughly proportional to the number of individuals that have the corresponding haplotype. Transitions between haplotypes are denoted with the polymorphic site number.

## IV.3.6.2 Genetic differentiation between populations

An exact test of population subdivision revealed significant differentiation between all populations for comparisons done for both the all and *restricted* datasets ( $p$ -value =  $0.000 \pm 0.000$ ), with the exception of the MED-NSEA ( $p$ -value =  $0.049 \pm 0.001$ ) ‘*restricted*’ comparisons (Table IV.5). After correction with sequential Bonferroni, the MED-NSEA and the MED-NAO ‘*restricted*’ datasets were non-significant, suggesting no differentiation between the two. This result may be a consequence of the ‘*restricted*’ dataset’s small sample size ( $N=7$ ) for the MED.

**Table IV.5** Exact test  $p$ -values for population comparisons. Datasets are divided into all and *restricted* material.  $P$ -values significant at the 0.05 level after sequential Bonferroni correction are in bold type and marked with an ‘\*’.

	Material	$N$	GOM	MED	NSEA
GOM	all	96	—	—	—
	<i>restricted</i>	40			
MED	all	19	$0.000 \pm 0.000$	—	—
	<i>restricted</i>	7	$0.000 \pm 0.000$		
NSEA		18	$0.000 \pm 0.000$	$0.000 \pm 0.000$	—
		16	$0.000 \pm 0.000$	<b><math>0.049 \pm 0.001</math>*</b>	
NAO	all	86	$0.000 \pm 0.000$	$0.000 \pm 0.000$	—
	<i>restricted</i>	67	$0.000 \pm 0.000$	<b><math>0.041 \pm 0.002</math>*</b>	

$F_{ST}$  and  $\Phi_{ST}$  measures of genetic differentiation were calculated for the sequenced fragment based on conventional  $F$ -statistics and the Tamura-Nei (gamma = 0.5) distance measures respectively. A comparison between the GOM, MED and NSEA populations was performed first. A second comparison between the GOM, MED and NAO (which combines the sequences from the NSEA and whales throughout the NAO into an overall NAO population) was then performed. AMOVA results, incorporating  $\Phi_{ST}$ , suggest that the primary source of variation (60.54% / 59.55%) for the GOM, MED and NSEA ‘all’ and ‘*restricted*’ samples originates within populations and 39.46% / 40.55% of the variation is attributed to among population variation. As expected, similar results were obtained when we examined the within population variation (59.31% / 57.77%) and among population variation (40.69% / 42.33%) for both datasets in the GOM, MED and NAO

comparison. All pairwise calculated values, apart from for the MED-NSEA ‘restricted’ population comparison, proved highly significant ( $p < 0.01$ ) for both  $F_{ST}$  and  $\Phi_{ST}$  genetic differentiation measures (Table IV.6). After correcting for multiple comparisons using the sequential Bonferroni correction, the MED-NSEA ‘restricted’ comparison was no longer significant at  $p < 0.05$ . Overall, rather high  $F_{ST}$  and  $\Phi_{ST}$  values showed extensive differentiation between the GOM, NSEA, MED and NAO with respect to both the ‘all’ and ‘restricted’ population comparisons. The highest degree of differentiation (using the  $\Phi_{ST}$  measure) was between the MED and NSEA ‘all’ dataset ( $\Phi_{ST} = 0.485$ ,  $p < 0.001$ ) and the lowest of the  $\Phi_{ST}$  values was for the MED-NAO ‘restricted’ comparison ( $\Phi_{ST} = 0.333$ ,  $p < 0.01$ ).

**Table IV.6** mtDNA population comparison among three geographic areas (GOM, MED, and NAO).  $F_{ST}$  values are presented in the lower left matrix and  $\Phi_{ST}$  values for are presented in the upper right matrix. ‘Restricted’ dataset values are in italics and provided below the ‘all’ dataset values. Statistically significant p-values based on 10,000 permutations of the data and after Bonferroni corrections are marked with an asterisk ( $p < 0.05 = *$ ;  $p < 0.01 = **$ ;  $p < 0.001 = ***$ ).

	GOM	MED	NSEA	NAO
GOM	—	0.355*** <i>0.334**</i>	0.407*** <i>0.420***</i>	0.409*** <i>0.432***</i>
MED	0.539*** <i>0.511***</i>	—	0.485*** <i>0.365</i>	0.355*** <i>0.333**</i>
NSEA	0.339*** <i>0.326***</i>	0.443*** <i>0.319</i>	—	—
NAO	0.318*** <i>0.292***</i>	0.310*** <i>0.279**</i>	—	—

#### IV.3.6.3 Genetic distance between populations

$D_a$ , the extent of nucleotide divergence occurring between populations, was extremely low for the ‘all’ and ‘restricted’ datasets (range = 0.001 to 0.003) (Table IV.7). The lack of separation in values seen between the MED and the GOM was interesting given the geographic distance separating the two being much greater than comparisons among samples collected from the MED, NSEA and NAO populations.

**Table IV.7** Nei’s  $D_{xy}$  and  $D_a$  genetic distance measurements between the GOM, MED, NSEA and NAO.  $D_a$  values are presented in the lower left matrix and  $D_{xy}$  values for are presented in the upper right matrix. The ‘*restricted*’ dataset for each comparison is in italics.

	GOM	MED	NSEA	NAO
GOM	—	0.004 / 0.003	0.004 / 0.004	0.004 / 0.005
MED	0.002 / 0.003	—	0.003 / 0.003	0.003 / 0.003
NSEA	0.002 / 0.002	0.001 / 0.001	—	—
NAO	0.002 / 0.002	0.001 / 0.001	—	—

### IV.3.7 Microsatellite Results

All population differentiation analyses using nuclear DNA ‘microsatellite’ markers between the GOM, MED and the NSEA were conducted on two sets of data – ‘all’ and ‘*restricted*’.

#### IV.3.7.1 Microsatellite genetic diversity within populations

##### IV.3.7.1.1 Linkage disequilibrium

Sixteen microsatellite loci were analyzed for 125 individuals from three different geographic locations. Linkage disequilibrium was tested for each pair of loci within each of the three putative geographic populations and across the data as a whole to ensure that loci were independent of one another. Eleven locus pairs in the ‘all’ dataset (GOM = 9, MED = 1, NSEA = 1) showed linkage disequilibrium with a  $p$ -value < 0.05. After correcting the given  $p$ -values with the sequential Bonferroni adjustment for multiple comparisons, the resulting  $p$ -values were non-significant for the ‘all’ population; therefore no association between alleles at different loci was assumed in subsequent analyses.

##### IV.3.7.1.2 HWE deviation

Estimations of HWE deviation were performed for each population at each locus by testing for heterozygote deficiency and excess within populations (Table IV.8) and on a global scale. After sequential Bonferroni adjustments were made to the existing  $p$ -values, significant deviation ( $p < 0.05$ ) from HWE with respect to heterozygosity

**Table IV.8** Testing for deviation from HWE. Significant *p*-values before sequential Bonferroni corrections are listed. *P*-values that remained significant after Bonferroni corrections are in bold type and marked with an asterisk. A '-' indicates that the test was not performed for locus D08 due to all individuals being homozygous at the same allele in this dataset.

Locus	Heterozygote Deficiency <i>p</i> -values					
	Gulf of Mexico		Mediterranean Sea		North Sea	
	All	Restricted	All	Restricted	All	Restricted
EV1	0.1070	0.1417	1.0000	1.0000	0.6884	0.6234
EV5	0.1730	0.5106	0.8390	1.0000	0.0268	0.0187
EV37	0.0231	<b>0.0048*</b>	<b>0.0000*</b>	<b>0.0013*</b>	0.8970	1.0000
EV94	0.4787	0.9562	0.8547	0.5100	0.1653	0.1698
EV104	<b>0.0063*</b>	0.0222	<b>0.0005*</b>	<b>0.0054*</b>	0.4801	0.3256
SW10	0.9978	0.9863	0.3468	1.0000	0.1639	0.1380
SW13	0.8198	0.7853	0.8581	0.5897	0.8419	0.7553
SW19	0.5966	0.8501	0.2810	0.1270	0.0679	0.0482
TEXVET5	0.5883	0.6722	0.2747	0.7637	0.4658	0.5213
D08	0.0354	0.0262	0.2907	-	0.3331	0.3083
D22	0.1544	0.1031	0.8888	0.6420	0.7663	0.8915
FCB1	0.9621	0.9330	0.8703	0.5803	0.9109	0.7716
FCB14	0.9531	0.7902	0.9411	1.0000	0.3399	0.2967
FCB17	0.5131	0.2748	0.3033	1.0000	0.0360	0.0489
GATA28	0.3946	0.0492	0.5410	0.7016	0.5780	0.6020
GATA417	0.5091	0.5565	0.4432	1.0000	0.2876	0.3745

deficiency remained at locus EV37 (GOM 'restricted', MED 'all' and MED 'restricted' datasets) and EV104 for the GOM 'all' and the MED 'all' and 'restricted' datasets. Similar results were obtained with GENEPOP 3.2's global comparison test.

#### IV.3.7.1.3 Microsatellite allele frequency variation among geographic populations

Under HWE, the observed and expected heterozygosity should be similar in value. Discrepancy between the two may be a result of non-random mating or inbreeding, null alleles or the Wahlund effect. Mean observed and expected levels of heterozygosity over all 16 loci across all 6 datasets are shown in Table IV.9.

**Table IV.9** Mean observed and expected heterozygosity levels for each population's respective dataset.

Heterozygosity	Gulf of Mexico		Mediterranean Sea		North Sea	
	All	Restricted	All	Restricted	All	Restricted
$H_O$	0.742	0.750	0.651	0.648	0.742	0.741
	$\pm 0.162$	$\pm 0.186$	$\pm 0.240$	$\pm 0.314$	$\pm 0.158$	$\pm 0.155$
$H_E$	0.752	0.759	0.687	0.671	0.762	0.766
	$\pm 0.133$	$\pm 0.143$	$\pm 0.211$	$\pm 0.258$	$\pm 0.142$	$\pm 0.145$

Individual locus observed heterozygosity values were compared and the majority showed similar levels of variation across populations with values ranging from 0.273 to 0.964 and 0.000 to 1.000 (Table IV.10). The extreme low and high values of  $H_O$  are most likely a direct result of the low sample sizes obtained for the MED and NSEA datasets.

The results of testing for null allele proportions within populations using the program CERVUS 2.0 are shown in Table IV.10. Due to the MED 'restricted' datasets small sample size ( $n=8$ ), null allele proportions could not be estimated. Null allele estimates incorporating all locations in the 'all' and 'restricted' datasets showed high proportions of null alleles expected to occur at locus EV37 (+0.076/+0.064), EV104 (+0.094/+0.089) and D08 (+0.117/+0.153). Null allele proportions varied within individual populations and datasets, although the same three loci were generally at high levels in the GOM and MED (EV37 = 0.339) datasets and the most likely to contribute null alleles to the results. The thirteen other loci had overall null allele frequencies below 0.05. The small sample sizes for both the MED and NSEA are most likely influencing the large null allele estimates observed at particular loci. For population comparison purposes, tests of differentiation (e.g.  $F_{ST}$ ,  $R_{ST}$  and  $Rho_{ST}$ ) were run with and without EV37, EV104 and D08 as a means of certainty. All population comparison tests performed without the three loci showed similar results with similar  $p$ -values when these loci were included and excluded in the population datasets (data not shown). No loci were eliminated as a result of deviation from HWE due to the fact that departure from HWE may not be a direct consequence of linked alleles, the presence of null alleles, or bogus amplification results; but rather an indication of population subdivision or non-random mating (i.e. inbreeding).

**Table IV.10** Genetic diversity for each population and each microsatellite locus: N = number of individuals; k = number of alleles; All. Rich = allelic richness;  $H_O$  = observed heterozygosity;  $H_E$  = expected heterozygosity;  $F_{IS}$  = Wright's inbreeding coefficient; Null freq. = test for null alleles; (?) = test could not be performed due to small sample size).

Locus	Location & Dataset	N	k	All. Rich.	$H_O$	$H_E$	$F_{IS}$	Null freq.
EV1	GOM 'All'	83	8	5.999	0.530	0.564	0.061	0.029
	GOM 'Restricted'	40	8	4.647	0.650	0.666	0.024	-0.012
	MED 'All'	22	4	3.716	0.273	0.253	-0.082	-0.065
	MED 'Restricted'	8	2	1.993	0.375	0.325	-0.167	?
	NSEA 'All'	20	8	7.231	0.650	0.599	-0.088	-0.076
	NSEA 'Restricted'	18	8	4.204	0.667	0.621	-0.077	-0.073
EV5	GOM 'All'	83	9	6.932	0.651	0.704	0.077	0.034
	GOM 'Restricted'	40	9	5.315	0.825	0.782	-0.055	-0.043
	MED 'All'	22	6	5.771	0.864	0.793	-0.092	-0.054
	MED 'Restricted'	8	6	5.642	1.000	0.858	-0.179	?
	NSEA 'All'	20	7	6.550	0.650	0.749	0.135	0.052
	NSEA 'Restricted'	18	7	4.693	0.611	0.751	0.191	0.085
EV37	GOM 'All'	83	22	12.874	0.831	0.911	0.088	0.043
	GOM 'Restricted'	40	18	7.979	0.800	0.917	0.129	0.063
	MED 'All'	22	11	10.516	0.429	0.887	0.523	0.339
	MED 'Restricted'	8	7	5.992	0.375	0.842	0.571	?
	NSEA 'All'	20	15	13.905	0.950	0.903	-0.054	-0.039
	NSEA 'Restricted'	18	14	7.956	1.000	0.913	-0.099	-0.063
EV94	GOM 'All'	83	13	8.742	0.831	0.809	-0.028	-0.016
	GOM 'Restricted'	40	13	6.502	0.925	0.857	-0.081	-0.046
	MED 'All'	22	8	7.536	0.909	0.847	-0.076	-0.047
	MED 'Restricted'	8	6	5.678	0.875	0.867	-0.010	?
	NSEA 'All'	20	10	9.380	0.750	0.844	0.114	0.046
	NSEA 'Restricted'	18	10	6.410	0.778	0.865	0.104	0.039



**Table IV.10 (cont.)** Genetic diversity for each population and each microsatellite locus: N = number of individuals; k = number of alleles; All. Rich = allelic richness;  $H_O$  = observed heterozygosity;  $H_E$  = expected heterozygosity;  $F_{IS}$  = Wright's inbreeding coefficient; Null freq. = test for null alleles; (?) = test could not be performed due to small sample size).

Locus	Location & Dataset	N	k	All. Rich.	$H_O$	$H_E$	$F_{IS}$	Null freq.
<b>EV104</b>	GOM 'All'	83	4	3.999	0.614	0.723	0.150	0.076
	GOM 'Restricted'	40	4	3.809	0.600	0.718	0.166	0.076
	MED 'All'	22	4	3.967	0.381	0.669	0.437	0.262
	MED 'Restricted'	8	4	3.700	0.250	0.692	0.654	?
	NSEA 'All'	20	5	4.700	0.700	0.697	-0.004	-0.019
	NSEA 'Restricted'	18	5	3.628	0.667	0.683	0.024	-0.021
<b>SW10</b>	GOM 'All'	83	11	9.490	0.964	0.873	-0.105	-0.055
	GOM 'Restricted'	40	11	6.781	0.975	0.879	-0.111	-0.060
	MED 'All'	22	9	8.713	0.818	0.869	0.060	0.022
	MED 'Restricted'	8	8	6.893	1.000	0.875	-0.155	?
	NSEA 'All'	20	10	9.902	0.900	0.885	-0.018	-0.027
	NSEA 'Restricted'	18	10	7.106	0.889	0.889	0.000	-0.023
<b>SW13</b>	GOM 'All'	83	10	7.829	0.831	0.803	-0.035	-0.018
	GOM 'Restricted'	40	10	5.722	0.875	0.812	-0.078	-0.046
	MED 'All'	22	5	4.725	0.636	0.582	-0.095	-0.046
	MED 'Restricted'	8	4	3.650	0.500	0.525	0.051	?
	NSEA 'All'	20	8	7.696	0.900	0.817	-0.105	-0.070
	NSEA 'Restricted'	18	8	5.803	0.889	0.830	-0.073	-0.054
<b>SW19</b>	GOM 'All'	83	17	12.434	0.916	0.907	-0.010	-0.009
	GOM 'Restricted'	40	16	8.054	0.950	0.920	-0.034	-0.022
	MED 'All'	22	9	8.271	0.818	0.851	0.040	0.006
	MED 'Restricted'	8	5	4.735	0.625	0.817	0.247	?
	NSEA 'All'	20	13	12.359	0.800	0.917	0.130	0.057
	NSEA 'Restricted'	18	13	7.879	0.778	0.917	0.156	0.070

**Table IV.10 (cont.)** Genetic diversity for each population and each microsatellite locus: N = number of individuals; k = number of alleles; All. Rich = allelic richness;  $H_O$  = observed heterozygosity;  $H_E$  = expected heterozygosity;  $F_{IS}$  = Wright's inbreeding coefficient; Null freq. = test for null alleles; (?) = test could not be performed due to small sample size).

Locus	Location & Dataset	N	k	All. Rich.	$H_O$	$H_E$	$F_{IS}$	Null freq.
<b>TEXVET5</b>	GOM 'All'	83	11	8.616	0.843	0.833	-0.012	-0.011
	GOM 'Restricted'	40	11	5.997	0.875	0.845	-0.036	-0.025
	MED 'All'	22	7	6.903	0.773	0.834	0.075	0.028
	MED 'Restricted'	8	6	5.599	0.875	0.842	-0.043	?
	NSEA 'All'	20	9	8.529	0.850	0.847	-0.003	-0.016
	NSEA 'Restricted'	18	8	5.796	0.833	0.849	0.019	-0.004
<b>D08</b>	GOM 'All'	83	5	4.356	0.530	0.624	0.151	0.098
	GOM 'Restricted'	40	5	3.412	0.450	0.572	0.216	0.144
	MED 'All'	21	4	3.777	0.286	0.336	0.152	0.140
	MED 'Restricted'	7	1	1.000	0.000	0.000	-	?
	NSEA 'All'	19	5	4.789	0.474	0.559	0.156	0.102
	NSEA 'Restricted'	17	5	3.417	0.471	0.545	0.141	0.081
<b>D22</b>	GOM 'All'	83	6	4.358	0.530	0.573	0.076	0.039
	GOM 'Restricted'	40	4	3.277	0.425	0.514	0.174	0.097
	MED 'All'	20	4	3.850	0.750	0.650	-0.160	-0.085
	MED 'Restricted'	8	3	2.992	0.625	0.625	-0.000	?
	NSEA 'All'	20	5	4.829	0.550	0.518	-0.064	-0.031
	NSEA 'Restricted'	18	4	3.209	0.556	0.494	-0.130	-0.078
<b>FCB1</b>	GOM 'All'	83	11	8.897	0.867	0.815	-0.064	-0.035
	GOM 'Restricted'	40	9	5.949	0.850	0.807	-0.054	-0.028
	MED 'All'	22	8	7.762	0.909	0.857	-0.062	-0.039
	MED 'Restricted'	8	8	7.092	0.875	0.900	0.030	?
	NSEA 'All'	20	10	9.809	0.950	0.891	-0.068	-0.045
	NSEA 'Restricted'	18	10	7.026	0.944	0.889	-0.065	-0.046

**Table IV.10 (cont.)** Genetic diversity for each population and each microsatellite locus: N = number of individuals; k = number of alleles; All. Rich = allelic richness;  $H_O$  = observed heterozygosity;  $H_E$  = expected heterozygosity;  $F_{IS}$  = Wright's inbreeding coefficient; Null freq. = test for null alleles; (?) = test could not be performed due to small sample size).

Locus	Location & Dataset	N	k	All. Rich.	$H_O$	$H_E$	$F_{IS}$	Null freq.
<b>FCB14</b>	GOM 'All'	83	10	7.560	0.880	0.825	-0.066	-0.035
	GOM 'Restricted'	40	8	5.197	0.850	0.808	-0.053	-0.032
	MED 'All'	17	7	7.000	0.941	0.848	-0.113	-0.068
	MED 'Restricted'	6	6	6.000	1.000	0.864	-0.177	?
	NSEA 'All'	20	8	7.662	0.800	0.831	0.038	0.006
	NSEA 'Restricted'	18	7	5.336	0.778	0.829	0.063	0.018
<b>FCB17</b>	GOM 'All'	83	20	13.923	0.904	0.915	0.012	0.004
	GOM 'Restricted'	40	19	8.242	0.875	0.922	0.052	0.022
	MED 'All'	22	8	7.659	0.727	0.765	0.051	0.012
	MED 'Restricted'	8	6	5.199	1.000	0.767	-0.333	?
	NSEA 'All'	20	15	14.153	0.850	0.924	0.082	0.027
	NSEA 'Restricted'	18	15	8.436	0.833	0.929	0.105	0.039
<b>GATA28</b>	GOM 'All'	83	3	3.000	0.639	0.637	-0.003	-0.008
	GOM 'Restricted'	40	3	2.916	0.575	0.637	0.098	0.029
	MED 'All'	22	3	3.000	0.591	0.606	0.025	0.004
	MED 'Restricted'	8	3	2.750	0.625	0.592	-0.061	?
	NSEA 'All'	20	3	3.000	0.600	0.617	0.028	0.005
	NSEA 'Restricted'	18	3	2.887	0.611	0.624	0.021	0.005
<b>GATA417</b>	GOM 'All'	83	4	3.361	0.518	0.518	0.000	-0.003
	GOM 'Restricted'	40	3	2.672	0.500	0.497	-0.006	-0.017
	MED 'All'	22	3	2.952	0.318	0.348	0.087	0.021
	MED 'Restricted'	8	3	2.700	0.375	0.342	-0.105	?
	NSEA 'All'	20	4	3.850	0.500	0.596	0.165	0.085
	NSEA 'Restricted'	18	4	3.152	0.556	0.624	0.112	0.051

Allele sizes and frequencies at each locus within each of the three geographic populations are presented in Appendix II. Total alleles across all three populations for both the 'all' and 'restricted' datasets was 174 and 163 respectively. The GOM 'all' and 'restricted' datasets contained the largest number of scored alleles (total alleles = 164 and 151 respectively), while the MED showed the lowest amount (total alleles = 100 and 78 respectively). This result is most likely directly attributed to the difference in sample sizes between geographic areas. Allele sizes didn't appear to differ drastically between populations and were similar to values produced by both Lyrholm et al. (1999) and Bond (1999). Several loci exhibited higher levels of polymorphism than others in both the 'all' (allele range = 3–24 alleles/locus) and 'restricted' (allele range = 3–21 alleles/locus) datasets and the mean number of alleles/locus over all sixteen loci across all individuals in all populations for each dataset was 10.88 and 10.19 respectively. Values of allelic richness are provided in Table IV.10. EV37 was the most polymorphic of the utilized loci with a total of 24 alleles. Amplified with primers developed for balaenopterid whales (Palsboll et al 1997a), GATA028 was the least variable of the loci with only three alleles scored in each population. Forty-three (24.7%) of the 174 alleles scored in the 'all' dataset and 33 (20.2%) of the 163 alleles scored in the 'restricted' dataset were private alleles (alleles only occurring in a particular population). The percentage of private alleles ( $pa$ ) observed per population and per dataset was as follows: GOM ( $pa$  = 19.0%, 15.3%); MED ( $pa$  = 0.5%, 0.0%); and NSEA ( $pa$  = 5.2%, 4.9%). Overall, private alleles occurred more frequently within the GOM population, but at rather low frequencies across all three populations and both datasets ( $pa \leq 0.078$  and  $pa \leq 0.075$  respectively) (Appendix II).  $F_{IS}$  values were based on observed and expected heterozygosity levels for each locus within each of the six 'all' and 'restricted' population datasets (Table IV.10). Large positive values of  $F_{IS}$  at particular loci over multiple populations can be an indicator of homozygosity excess at that locus. Positive  $F_{IS}$  values (range = 0.012 to 0.654) were seen for loci EV37, EV104, SW19, D08, FCB17 and GATA28 at a minimum of four of the six tested datasets. However, loci SW19, D08, FCB17 and GATA28 all appeared to be in HWE for all populations and datasets.

#### IV.3.7.2 Population genetic differentiation

##### IV.3.7.2.1 Allelic and genotypic distributions

An exact test used to test allelic distribution in different populations (null hypothesis: that allelic distribution is identical across populations) yielded interesting results with respect to population differentiation. However, results may be due to low power at some loci. The extent of significant genetic differentiation between putative populations depended on the locus that was screened. Comparisons for each 'all' dataset population pair across all loci using Fisher's method revealed highly significant  $p$ -values ( $p < 0.001$ ) for both the GOM-MED and the MED-NSEA. Comparisons for each '*restricted*' dataset population pair across all loci using Fisher's method revealed significant  $p$ -values ( $p < 0.01$ ) for both the GOM-MED and the MED-NSEA.

Tests of genotypic differentiation (null hypothesis: the genotypic distribution is identical across populations) were also performed and the results confirmed several of the previous genetic results. Several loci that differentiated pairs of populations in both datasets continued to differentiate them at significant levels ( $p < 0.05$  after seq. Bonferroni correction). Comparisons for each 'all' dataset population pairs across all loci using Fisher's method revealed highly significant  $p$ -values ( $p < 0.001$ ) for both the GOM-MED and the MED-NSEA before and after sequential Bonferroni corrections. Comparisons for each '*restricted*' dataset population pair across all loci using Fisher's method revealed significant  $p$ -values ( $p < 0.01$ ) for both the GOM-MED and the MED-NSEA before and after sequential Bonferroni corrections.

##### IV.3.7.2.2 Population structure

The extent of genetic differences that lead to genetic structuring between populations was tested using  $F_{ST}$ , theta ( $\theta$ ),  $R_{ST}$  and  $Rho_{ST}$  statistical measures. Each measures the extent of genetic variation that can be attributed to the genetic differences between each population pair.  $R_{ST}$  (Slatkin 1995) and  $Rho_{ST}$ , the latter being an unbiased analogue to  $R_{ST}$  developed by Goodman (1997), were developed specifically for microsatellites and take the SMM model into account when calculating differentiation. Based on previous sperm whale genetic and behavioural

findings, our null hypothesis was that variation observed in nuclear markers (i.e. microsatellites) between putative populations would be low or non-existent, a result that is suggestive of male dispersal between populations.

$F_{ST}$  and  $\theta$  estimates for the GOM-MED and the MED-NSEA comparisons each showed low, but significant ( $p < 0.01$ ), differentiation between populations in the ‘all’ and ‘restricted’ datasets before and after sequential Bonferroni corrections were implemented (Table IV.11). Pairwise results for  $R_{ST}$  and  $Rho_{ST}$  are also provided in Table IV.11.  $Rho_{ST}$  over all populations was 0.020 (95% CI: 0.018 – 0.058;  $p < 0.01$ ) and 0.014 (95% CI: 0.013 – 0.083;  $p > 0.05$ ) for the ‘all’ and ‘restricted’ datasets respectively.

**Table IV.11** Pairwise comparisons and distance measurements for the GOM, MED and NSEA populations using  $F_{ST}$ ,  $\theta$ ,  $R_{ST}(S)$ ,  $Rho_{ST}$  and  $\delta\mu^2$ . ‘Restricted’ dataset values are in italics and provided below the ‘all’ dataset values. Statistically significant  $p$ -values after Bonferroni corrections are marked with an asterisk ( $0.01 < p < 0.05 = *$ ;  $0.001 < p < 0.01 = **$ ;  $p < 0.001 = ***$ ).

	$F_{ST}$	$\theta$	$R_{ST}S$	$Rho_{ST}$	$\delta\mu^2$
GOM-MED	0.033***	0.035**	0.052***	0.037***	0.099
	<i>0.032***</i>	<i>0.037**</i>	<i>0.078**</i>	<i>0.047</i>	<i>0.166</i>
GOM-NSEA	0.000	0.000	0.009	0.004	0.041
	<i>-0.005</i>	<i>-0.005</i>	<i>0.015</i>	<i>0.004</i>	<i>0.050</i>
MED-NSEA	0.030***	0.030**	0.030	0.031	0.106
	<i>0.031**</i>	<i>0.033**</i>	<i>0.047</i>	<i>0.038</i>	<i>0.165</i>

Testing for a recent bottleneck event using the Wilcoxon test in the program BOTTLENECK 1.2.02 also showed some evidence of heterozygosity excess within the GOM population ( $p = 0.007$ ), but not the MED or NSEA populations ( $p = 0.058$ )

#### IV.3.7.3 Sex-biased dispersal

As previously described, genetic differentiation was highly significant among the GOM, MED and NSEA samples for the mtDNA control region sequences, but only the MED population showed significant differentiation for the microsatellite DNA loci. Sex-biased dispersal results are in agreement with females being the more

philopatric sex and males dispersing from their natal populations (Table IV.12). Although  $F_{ST}$  was slightly higher among females than males, it was non-significant ( $p > 0.05$ ). However, sperm whale population comparisons using nuclear markers show little to no significant differentiation with respect to nuclear DNA and this may

**Table IV.12** Sex-biased dispersal results for males and females with respect to  $F_{IS}$ ,  $F_{ST}$ ,  $H_O$ ,  $H_S$ , mean assignment and variance assignment.

	$n$	$F_{IS}$	$F_{ST}$	$H_O$	$H_S$	Mean Assignment	Variance Assignment
Females	66	-0.004*	0.043	0.738	0.735*	0.785*	14.278
Males	59	0.049*	0.016	0.713	0.750*	-0.878*	16.010

\* $p$ -value  $< 0.01$

have a direct effect on  $F_{ST}$  testing for sex-biased dispersal. The variance assignment test provided an expected higher value for males (the dispersers) than for the females (the more philopatric sex), although values were slightly above the non-significant threshold ( $p = 0.081$ ). The mean assignment test,  $F_{IS}$  and  $H_S$  were all highly significant ( $p < 0.01$ ) and provided values in accordance with males dispersing.

#### IV.3.7.4 Genetic distance between populations

Genetic distances between populations were measured with the  $\delta\mu^2$  statistic.

Pairwise comparisons between populations and the resulting genetic distances (range = 0.050 to 0.166) are depicted in Table IV.11.

## IV.4 DISCUSSION

This chapter has focused on the assessment of genetic variation and differentiation within and between the GOM, MED, NSEA and NAO putative populations of sperm whales via the analysis of both mtDNA and nuclear genetic markers. The extent of variation and stock structure for each of the geographic regions (excluding the NAO comparison with the North Pacific and Southern Hemisphere ocean basins (Lyrholm and Gyllensten 1998; Lyrholm et al. 1999)) was unknown prior to this research.

These results add a significant contribution towards the proper management of sperm whale populations within geographic regions as well as provide a valuable addition to prior global scale sperm whale population genetic studies.

#### IV.4.1 Gender Composition

The majority (67.6%) of samples obtained in the GOM were from immature and adult female whales. The remaining 32.4% were from young males believed to be either immature or perhaps sexually mature, but not physically mature (less than 15 meters in length), based on rough visual size estimates using the length of the RHIB as a comparison. Immature male sperm whales are difficult to distinguish from adult females in the field so sampling preference to one sex over the other was not the case and random sampling with respect to gender was generally enforced. However, there were instances when 'larger' whales (believed to be males based on their size and semi-pronounced heads) were targeted for sampling. To date, no large physically mature (length >15 m (Best 1979)) males have been seen in the northern GOM during the past four years of sperm whale focused research cruises.

The MED samples were primarily collected from males (nearly 2:1). This result was most likely an artefact of unequal sampling effort and the sampling methods involved and does not imply that males occur more frequently than females in the MED.

All samples from the NSEA were from stranded whales believed to be immature or perhaps sexually mature males based on proper length measurements (Mean = 12.96 meters). North of these stranding locations is Bleikdjupet Canyon (near Andenes in the NSEA), an area where young males aggregate during the summer months (Lettevall et al. 2002) and this stranding event may have coincided with some form of southern migration from these waters.

#### IV.4.2 Variation Within and Between Localities – mtDNA

The 5' end of the mtDNA control region can be highly variable in many marine mammal species (see Hoelzel et al. 2002*b* for a review). In this examination of 399 bp of the mtDNA control region for 201 individual sperm whales, surprisingly low levels of nucleotide and haplotype diversity were found for each of the putative populations within each 'all' and 'restricted' datasets. Low levels of variation were also observed among the four populations with only six segregating sites defining 7 haplotypes throughout the entire study site spanning well over 12,000 km. The GOM possessed two unique haplotypes, one of which (haplotype 'X') was the most frequent (58%) within the population. Four haplotypes ('Lyr4', 'N', 'X' and 'Y')



were unique to specific geographic populations ('Lyr4' and 'N' were only found in the NAO; 'X' and 'Y' were exclusive to the GOM). Although haplotypes 'Lyr4' and 'N' comprised less than 8% of the NAO makeup, haplotypes 'X' and 'Y' comprised approximately 68% of the total haplotypes found in the GOM datasets.

Haplotype and nucleotide diversity were low within ( $h$  range = 0.000 to 0.653;  $\pi$  range = 0.00% to 0.26%) and among geographic regions ( $h = 0.762$ ,  $\pi = 0.33\%$ ). The lack of variation in the mtDNA genome is not specific to sperm whales, nor is it common among cetacean species in general with low diversity found in narwhals, *Monodon monoceros*, ( $\pi=0.17\%$ ; Palsboll et al. 1997b), killer whales, *Orcinus orca*, ( $\pi=0.52\%$ ; Hoelzel et al. 1998b), northern bottlenose whales, *Hyperoodon ampullatus*, ( $h=0.57$ ,  $\pi=0.15\%$ ; Dalebout et al. 2001), and New Zealand North Island Hector's (Maui) dolphins, *Cephalorhynchus hectori*, ( $h=0.00$ ,  $\pi=0.00\%$ ; Pichler and Baker 2000) – see Hoelzel et al. 2002b for a comparative review among additional marine mammal species. While several cetacean species that are widely distributed exhibit high variability at this locus (e.g. humpback whales, *Megaptera novaeangliae*, ( $\pi=2.6\%$ ; Palsboll et al. 1995), and harbour porpoise, *Phocoena phocoena*, ( $\pi=1.1\%$ ; Rosel et al. 1999), sperm whales continue to show remarkably low levels of nucleotide diversity on a global scale ( $\pi=0.39\% \pm 0.03$ ) for the first 330 bp of the mtDNA control region (Lyrholm et al. 1996; Lyrholm and Gyllenstein 1998). While diversity levels were approximately equivalent in the GOM, NSEA and NAO putative populations, the total lack of haplotype and nucleotide diversity ( $h = 0.00$ ,  $\pi = 0.00$ ) among samples collected within the MED implies some degree of population isolation, small effective population size or perhaps a reduction in maternal lineages brought on by a recent bottleneck event (Lyrholm and Gyllenstein 1998; Baker et al. 1999; Hoelzel 1999; Hoelzel et al. 2002a). A similar scenario in the MED appears to be present for fin whales. Berube et al. (1998) have shown fin whales within the MED to have low mtDNA diversity ( $\pi = 0.57\%$ , SE = 0.09) and the current assumption based on DNA, contaminant and observational evidence suggests that the MED may contain a separate stock from those in the NAO.

Several hypotheses have been put forward to explain the lack of intraspecific mtDNA genetic diversity in the sperm whale. Lyrholm et al. (1996) addressed questions regarding a lower substitution rate occurring in the mtDNA control regions of sperm whales vs. other species and provided evidence that there was no indication

of this occurring. The second hypothesis by Whitehead (1998) suggests that low mtDNA diversity in species such as sperm whales, pilot whales and killer whales may be a direct result of matrilineal based social structure. Whitehead's theory is based on the molecular "hitchhiking" concept where diversity at a neutral locus (in this case the maternally inherited mtDNA control region) is reduced via selection acting on a linked locus. Whitehead (1998) suggests that selected maternally inherited cultural (culture being defined as: information or behaviour acquired from conspecifics by imitation or learning (Boyd and Richerson 1985)) traits may act as a means for neutral or nearly neutral mtDNA loci to hitchhike, thus reducing the level of mtDNA diversity. Cultural transmission from mothers to offspring within their matrilineal group also seems plausible in the case of two forms of orca, where the young of 'fish eating' (a.k.a. 'residents') and 'mammal eating' (a.k.a. 'transients') foraging specialists learn specific feeding techniques from their mothers within highly stable groups and show clear signs of morphological, behaviour, social structure and genetic variation between different specialist communities occupying similar habitats (Hoelzel et al. 1998b; Baird 2000; Hoelzel et al. 2002c). Whitehead provides an exciting concept, however, the theory relies on two assumptions that are unlikely to be fulfilled: 1) the stipulation that transmission is strictly matrilineal (i.e. transmitted traits are not learned by unrelated females that subsequently pass those traits on to her relatives) and that horizontal transmission will be at a rate below 0.5% and 2) it assumes that selection for cultural traits occurs in cetaceans. Mesnick et al. (1999) and Mesnick (2001) describe the genetic difficulties associated with this concept, primarily citing genetic evidence that sperm whale group structure is not purely matrilineal, nor are all whales within a group highly related maternally or paternally (see Chapter 5). In addition, Tajima's *D* and Fu's *F<sub>s</sub>* statistics indicates that the mtDNA control region does not appear to be under strong selection in sperm whales. However, such tests may have little power to detect selection (Wayne and Simonsen 1998). The third hypothesis by Tiedemann and Milinkovitch (1999) suggests that the reduction in mtDNA diversity for matrilineal whale populations like sperm whales may be a result of any stochastic heterogeneity in reproductive success through space and time. Finally, the hypothesis by Lyrholm et al. (1996) suggests that diversity within sperm whales may be directly linked to one or more past population bottlenecks events. Bottleneck effects among marine mammals may be

linked to selective sweeps or historical whaling/harvesting (Hoelzel et al. 1993; Lyrholm et al. 1996; Baker et al. 1999; Hoelzel et al. 2002a). Molecular evidence for a severe population bottleneck event brought on by human exploitation towards the end of the nineteenth century was recently provided for the northern elephant seal (*Mirounga angustirostris*) (Hoelzel 1993; Hoelzel 1999; Hoelzel et al. 2002a). By measuring genetic diversity between archive samples collected pre and post hypothesized bottleneck period, they were able to provide evidence for a loss of variation and a disruption in the pattern of allele frequencies (post-bottleneck) had indeed transpired. One plausible cause for the loss of variation among sperm whales may be linked to the extent of whaling ground decimation by whalers primarily in the twentieth century. According to Whitehead's calculations, worldwide pre-whaling sperm whale estimates were approximately one million whales. Post-whaling (current) estimates suggest a global population size of 32% of the pre-whaling level (i.e. 320,000) with much of the population declining dramatically between 1945 and 1975 (Whitehead 2002). Although not all areas containing sperm whales were heavily decimated, the extent of whaling ground decimation catapulted by modern whaling during the twentieth century may be a factor in decreasing genetic differentiation with respect to mtDNA between modern-day populations. In comparison, two southern right whales (*Eubalaena australis*) wintering grounds showed significantly reduced haplotype diversity, perhaps as a direct result of intensive whaling (Baker et al. 1999). A similar comparison based on methods described by Hoelzel et al.'s (2002a) study for northern elephant seals would provide a better understanding as to what extent mtDNA variation may have been lost between historical (pre-whaling) and modern day sperm whale samples taken from distinct populations. However, the current post-whaling global sperm whale estimates provided by Whitehead (2002) were not as drastically reduced as these other species, so variability between populations shouldn't be as affected. Time since common mtDNA ancestry estimates indicate a relatively young global population structure with an age of *ca.* 24,000 – 92,000 years at most (Lyrholm et al. 1996; Lyrholm and Gyllenstein 1998). This relatively recent (in an evolutionary timeframe) coincides with the last of the major ice ages (the Pleistocene Epoch). Population ranges may have been restricted in relation to prey and habitat availability during this time as a direct result of the Pleistocene glaciation era that

lasted up until 10,000 years ago (Lyrholm et al. 1996; Lyrholm and Gyllensten 1998). Population expansion towards the end of this ice age may have significantly contributed to the low levels of diversity seen today among sperm whale populations. A suite of additional factors exhibited by sperm whales including site-fidelity by matrilineal based female groups (containing genetically related whales and multiple matrilines in some cases) (Richard et al. 1996a; Christal 1998; Lyrholm and Gyllensten 1998; Whitehead et al. 1998), relatively low calving rates (Best 1979), longevity among whales (Best 1979; Rice 1989), long-term stable associations between females within groups, a difference in male and female dispersal patterns and group and clan specific dialects (Weilgart and Whitehead 1997; Rendell and Whitehead 2003) may have contributed to the overall impact of a proposed bottleneck event (Lyrholm et al. 1996; Lyrholm and Gyllensten 1998). Results from this study's mismatch distributions for the control region may suggest a unimodal distribution implying expansion, but the resolution is quite low due to the small number of haplotypes present and not as distinguishable as those recently described for orca (Hoelzel et al. 2002c). BOTTLENECK 1.2.02 calculations also showed some evidence for a recent bottleneck event, seen as a significant heterozygosity excess in the GOM population. However, Tamura's  $D$  and Fu's  $F_s$  are not in agreement with a bottleneck event. Instead of one underlying factor that has influenced sperm whale mtDNA diversity; a more probable scenario is perhaps a combination of multiple events mentioned above.

#### IV.4.3 Population Structuring – mtDNA

Various types of data including dialects, genetics, mark-recapture data, morphology, parasitism and predation (Best 1979; Rice 1989; Lyrholm and Gyllensten 1998; Whitehead et al. 1998; Lyrholm et al. 1999) suggest female philopatry in sperm whales, while adult males are known to be capable of ranging over vast distances (Best 1979; Rice 1989; Whitehead and Weilgart 2000). Consistent with this, the comparison of mtDNA haplotypes between regions proved highly significant with respect to population structuring. While the extremely low nucleotide variation in sperm whales are reflected in low  $D_a$  genetic distance estimates, exact tests revealed strong differentiation between each population pairwise comparison for the 'all' and all but two (MED-NSEA and the MED-NAO) of the 'restricted' datasets. The

MED-NSEA and the MED-NAO comparisons were only marginally non-significant after sequential Bonferonni corrections. Pairwise comparisons for  $F_{ST}$  and  $\Phi_{ST}$  measures of genetic differentiation also supported exact test results with highly significant findings for all population comparisons across both measures ( $F_{ST}$  range = 0.279 to 0.539;  $\Phi_{ST}$  range = 0.333 to 0.485). Lyrholm and Gyllensten (1998) results are consistent with our findings, providing evidence of mitochondrial genetic differentiation on a world-wide scale. However, their NAO results were only compared over large scales (i.e. between oceans on a global scale). This study incorporates the sequences from the Lyrholm and Gyllensten (1998) NAO population study into a more detailed comparison of multiple putative populations in neighbouring geographic localities. Gene flow estimates ( $Nm$ ) were not performed on the datasets due to the potential for this  $F_{ST}$ -based estimate of migration to be misleading and inaccurate (Neigel 2002; Beerli 2004; Pearse and Crandall, in press). The extent of genetic structuring based on the maternally inherited mtDNA genome described here is in agreement with the current theory suggesting significant female philopatry and male dispersal.

#### IV.4.4 Variation Within and Among Localities – Nuclear DNA

$H_O$  levels within populations ranged from 65% to 74% for the 'all' dataset and 65% to 75% for the 'restricted' dataset. This relatively low level of  $H_O$  is not uncommon for sperm whales or other cetaceans. Lyrholm et al. (1999) showed average gene diversity across 10 loci for sperm whales in the NAO ranged from 0.57 to 0.88 and when examined on a global scale ranged from 0.47 to 0.91. Bond (1999) also showed the mean  $H_O$  across 12 loci for sperm whales in the Atlantic and Pacific to be 0.76 and 0.74 respectively. Similar gene diversity findings for multiple microsatellites comparisons across geographic populations have been seen for other marine mammals such as humpback whales ( $H_E = 0.691 - 0.745$  (Valsecchi et al. 1997)), killer whales ( $H_O = 0.00 - 0.72$  (Hoelzel et al. 2002c)) and fin whales ( $H_O = 0.42 - 0.83$  (Berube et al. 1998)). Although private alleles were present within populations (approx. 25% and 20% of the total number of alleles within the 'all' and 'restricted' datasets respectively), frequencies were below 8%. Genotypic disequilibrium was only evident for one pair of loci in the GOM 'restricted' dataset. This may be a result of population substructure. Krutzen (2002) showed similar

disequilibrium findings when he compared bottlenose dolphin population structure off Australia. However, the GOM '*restricted*' dataset had the largest number of samples for all three '*restricted*' datasets. If linkage were present between the two loci in this putative population, then one would expect to find similar results in other populations with lower sample sizes (i.e. the MED and the NSEA), which we did not. The possible presence of null alleles for EV37, EV104 and D08 and the resulting effect they may have on calculations proved difficult to determine due to low sample sizes in the MED and NSEA populations. Similar high null allele frequency results for EV37 and EV104 were also found by Bond (1999) in the Atlantic and Pacific comparisons. All three loci are derived from different cetacean species (EV37 and EV104 are from humpback whales; D08 is from bottlenose dolphins) which may increase the probability of encountering null alleles. However, very few studies have thoroughly explored the origin of null alleles resulting in difficulties interpreting what happens in the case of cross-species amplifications and it seems unlikely that closely related species should drastically differ in their degrees of polymorphism (Schlotterer 1998). The lack of significant differences between population comparisons that included and excluded these three loci confirmed the assumption that even though loci may have high null allele frequencies, they do not necessarily have a strong effect on the degree of population structuring overall. Sampling of mother-calf pairs in future endeavours would help towards determining the presence or absence of null alleles.

#### IV.4.5 Population Structuring – Nuclear DNA

Genetic differentiation was assessed between three putative populations: the GOM, MED and the NSEA. Population subdivision was present for both the GOM-MED and the MED-NSEA 'all' and '*restricted*' datasets after comparing population pairs across all loci using Fisher's exact test method. Population differentiation based on exact tests,  $F_{ST}$ ,  $\theta$ ,  $R_{ST}$ ,  $Rho_{ST}$  and  $\delta\mu^2$  genetic distance measures showed a more contrasting pattern of population structuring than what was clearly visible using mtDNA markers.  $F_{ST}$ ,  $\theta$ ,  $R_{ST}$  and  $Rho_{ST}$  calculations showed significant ( $p < 0.01$ ) differentiation between seven of eight GOM-MED pairwise comparisons suggesting some degree of limited population structuring between pairs. The MED-NSEA 'all' and '*restricted*' datasets were significant for  $F_{ST}$  and  $\theta$ , but not  $R_{ST}$  or  $Rho_{ST}$ . By

using simulations of the SMM, Gaggiotti et al. (1999) concluded that  $F_{ST}$ -based estimates are always better than  $R_{ST}$  estimates when population sample sizes are small ( $N_s \leq 10$ ) and the number of loci used in comparisons is less than 20. In an ideal comparison involving large sample sizes ( $n_s \geq 50$ ) and twenty or more loci,  $R_{ST}$  performs better than  $F_{ST}$  (Gaggiotti et al. 1999). However,  $Rho_{ST}$  is provided to account for low sample sizes as well as the SMM model and should reflect a more accurate representation of differentiation than other measures. As a result of our low sample sizes for the MED and NSEA 'all' and 'restricted' populations and the relatively high number ( $N = 16$ ) of microsatellites used, the most conservative approach to indicate differentiation in this study's case would be to use  $F_{ST}$  and  $Rho_{ST}$ . The low  $F_{ST}$ ,  $R_{ST}$  and  $Rho_{ST}$  values ( $< 0.08$ ), although significant, indicate minimal genetic differentiation between the GOM-MED and the MED-NSEA (significant for  $F_{ST}$  only) populations with respect to nuclear differentiation. Bond (1999) found similar findings for fifteen sperm whale geographic populations with respect to differentiation using twelve microsatellite DNA markers. Bond's observed upper estimates of  $\theta$  (0.084) and  $R_{ST}$  (0.116) were nearly three times and two times respectively the size of the observed upper estimates found across this study's three populations. After Bonferroni correction for large comparison numbers, only one (Azores and New Zealand) of Bond's 225 population pair comparisons was significantly differentiated using the  $R_{ST}$  measure. However, low sample size ( $N \leq 9$ ) in nine of the fifteen 'geographic populations' may be influencing her results. When she compared the larger Atlantic Ocean with the Pacific Ocean, low but highly significant differentiation was seen for both  $F_{ST}$  (0.002) and  $R_{ST}$  (0.004). Lyrholm et al. (1999) has also shown similar results with respect to nuclear variation between sperm whale populations on a global scale. The Lyrholm et al. 1999 study utilized ten polymorphic microsatellites to determine and compare  $G_{ST}$  (Nei and Chesser 1983) and  $R_{ST}$  statistics across the NAO, Pacific Ocean and samples collected from the southern hemisphere. Both  $G_{ST}$  (0.001) and  $R_{ST}$  (0.005) were not significantly different from zero and indicated extremely low, if any, differentiation between oceans. Lyrholm et al. (1999) then compared allele frequencies for five sub-areas within the North Pacific (Galapagos Islands, Japanese coast, North Pacific western, central and eastern areas) and found no significant heterogeneity in allele frequencies (exact test;  $p = 0.392$ ). There is the possibility

that low sample sizes might bias population structure results. However, small sample sizes should generally cause population differences to be statistically non-significant, especially with respect to microsatellites (Waples 1998). Private alleles within regions or oceans were rare. The lack of differentiation between oceans leads one to suggest that males spread their genes to multiple populations of the more philopatric females, resulting in a reduction of nuclear differentiation on a global scale.

#### IV.4.6 Dispersal Behaviour

A variety of hypotheses suggest sex biases in mammalian dispersal may be related to advantages that occur for both males and females in competition for breeding resources or mates, or as a result of fecundity costs associated with dispersal between males and females (Greenwood 1980; Dobson 1982; Johnson 1986; Pusey 1987). Gender differences in dispersal can have a direct effect on how populations are structured (Avice 1994). Both male and female sperm whales are capable of very long-distance travel, however, female-based mix groups are thought to be more philopatric to particular lower-latitude waters, while males are believed to roam towards more polar waters in search of food as they age and grow (Rice 1989). Previous studies that compared the maternally inherited mtDNA and the biparentally inherited nuclear DNA strongly suggest that sex-biased dispersal for sperm whales is occurring on a global scale (Lyrholm et al. 1999).

This study's testing for sex-biased dispersal produced results that are in agreement with males being the dispersers and females exhibiting some degree of site-fidelity to particular geographic areas. The mean assignment test,  $F_{IS}$  and  $H_S$  were all highly significant ( $p < 0.01$ ) and in accordance with males being the dispersing sex. While  $F_{ST}$  results were higher for females than males, they were not significantly different. However, the lack of strong nuclear differentiation between population comparisons with respect to  $F_{ST}$  may be directly affecting our  $F_{ST}$  testing for sex-biased dispersal. The variance assignment test provided the expected higher value for the dispersers (males), although the  $p$ -value was slightly above the non-significant threshold ( $p = 0.081$ ). However, small sample sizes from individual populations can drastically affect the power of this test (Goudet et al. 2002)



These sex-biased dispersal results correspond with our highly significant mtDNA and generally non-significant nuclear DNA population structuring results. Various types of data (including dialects, genetics, mark-recapture data, morphology, parasitism, and predation (Best 1979; Rice 1989; Lyrholm and Gyllensten 1998; Whitehead et al. 1998; Lyrholm et al. 1999), including the data presented above, suggest female philopatry, while adult male sperm whales are known to be capable of ranging over vast distances (Best 1979; Rice 1989; Whitehead and Weilgart 2000).

#### **IV.4.7 Combining Techniques to Understand Population Structuring**

As previously stated, questions of population or stock structuring within cetaceans must be addressed using a variety of techniques that examine multiple parameters. The delineation of stock boundaries for endangered species is essential to estimate abundance, interpret life-history parameters, set catch limits, assess population changes, delineate critical habitats, verify catch or trade records, and establish territorial jurisdiction (Baker and Palumbi 1994; Baker et al. 1999). Our primary research area, the GOM, continues to combine an assortment of data from a wide array of scientific fields in order to better our understanding of how sperm whales utilize this important habitat. Each of these topics is briefly reviewed below.

Distribution and abundance estimate surveys over the last thirteen years and across seasons indicate that sperm whales, including some groups with calves, are reliably found over the northern GOM's continental slope near the Mississippi River Delta and in the south-eastern Gulf near the Dry Tortugas throughout the year (Davis et al. 1998; Waring et al. 2001). The resighting of whales within particular geographic areas in the northern GOM has lead some researchers to suggest that at least some whales maintain a level of intra and inter-annual site fidelity (Weller et al. 2000; Würsig et al. 2000).

Recent advances in technology allow the attachment of satellite-monitored radio transmitters (satellite-tags) to whales for long-term (several months) tracking purposes. As part of a multi-collaborative effort in the GOM, satellite-tags were attached to several sperm whales by Oregon State University (OSU) to provide an indication of preferential habitat use, site-fidelity and movement patterns through space and time. Tissue samples were obtained at the time of tag deployment for

genetic analyses and are included in the mtDNA population structuring results as well as the social structure results (see Chapter 5). Tag transmission results to describe both movement and association patterns are pending OSU's final assessment of the data.

The use of photoID techniques for a variety of marine mammals, sperm whales in particular (Christal and Whitehead 2001; Whitehead et al. 1998; Childerhouse et al. 1995), has been used with remarkable success to describe site-fidelity, association and movement patterns, habitat use and mark-recapture population estimates. The combination of photoID with genetic sampling techniques can allow identifiable individual sperm whales to contribute information towards group, unit and population structuring questions (Christal 1998; Bond 1999; Lyrholm et al. 1999). PhotoID studies have re-sighted known naturally marked individuals in the northern GOM over periods of days to years (Weller et al. 2000). Preliminary results indicate that sperm whales either display strong site fidelity to localized regions (e.g. the Mississippi River Canyon) or return to these particular regions on a frequent basis (Weller et al. 2000). For example, five whales were re-identified after a period of five or six years with distances between re-sightings ranging from 13 to 47 km (C. Cates, unpublished data).

Sperm whale groups appear to have distinctive dialects that may persist over periods of years (Weilgart and Whitehead 1997; Whitehead et al. 1998). The combination of genetic results with coda repertoires may provide clues towards assessing the discreteness of populations. Coda may also provide an indicator of cultural structure described by Whitehead et al. (1998) allowing the membership of larger clans, which may not be geographically structured, to be identified (Rendell and Whitehead 2001; Rendell and Whitehead 2003). Results from the examination of coda structure between the GOM and other geographic areas are still pending at this time.

Our genetic analyses of sperm whales inhabiting areas of the GOM, MED, NSEA and the NAO have begun to reveal valuable information regarding the degree of genetic variation between these putative populations of sperm whales. Degrees of gene flow are not easily measured for such a long ranging animal. By incorporating a suite of molecular marker techniques (molecular sexing, sixteen polymorphic microsatellites and mtDNA control region sequencing) into population assessments,

our results lend further support to both Lyrholm et al.'s (1999) and Whitehead and Weilgart's (2000) suggestions that females may exhibit long-term philopatry while sexually mature males rove between oceans and distribute their genes across multiple populations. This increase in male-mediated gene flow across oceans would clarify the lack of variation among nuclear DNA microsatellite markers, while the decrease in gene flow among philopatric females would help to explain the significant variation in the maternally inherited mtDNA marker. The accumulated evidence from the previously mentioned techniques, along with the data presented here on population structure using genetic markers, suggests that the northern Gulf of Mexico sperm whale stock should at the very least be classified as a separate stock from that of the North Atlantic Ocean stock. Additional differences such as sub-structure within stocks and the level of variation exhibited between other putative populations and the GOM (e.g. the Caribbean Sea, the southern Gulf of Mexico, the western and eastern North Atlantic Ocean) still require further study.

## ***V. Composition of Sperm Whale Groups and Clusters in the Northern Gulf of Mexico and the North Sea***

### **V.1 INTRODUCTION**

Sperm whales are most often found in mixed sex and all male (bachelor) groups (Best 1979; Whitehead and Arnborn 1987; Rice 1989). Mixed groups typically occur in lower latitude waters where females tend to band together and limit their large-scale movements (Best 1979; Arnborn et al. 1989; Whitehead et al. 1991). Sub-adult males eventually leave their natal groups, form loose aggregations with other young males and appear to migrate towards higher latitudes (Rice 1989). These young males tend to either pair up or become solitary after reaching both sexual and physical maturity. Upon reaching sexual and physical maturity, males return to lower latitudes to breed with females (Best 1979; Rice 1989; Whitehead 1993).

#### **V.1.1 Social Organization of Sperm Whales (Three Main 'Group' Types)**

##### ***V.1.1.1 Mixed sex social groups***

A mixed sex group is believed to be primarily matrifocal and comprised of adult females, calves and immatures of both sexes. Mixed groups are typically found in tropical and subtropical waters at latitudes between approximately 45° N to 40° S in the summer, and nearer the equator in the winter (Best 1979; Rice 1989). Whitehead and Kahn (1992) divided mixed groups into three levels of social structure (units, groups and aggregations). A fourth category is that of the cluster (Figure V.1) which is defined by Whitehead and Arnborn (1987) as whales within 100 m of one another that are swimming in a coordinated manner. Clusters are small scale associations that tend to last for brief durations (i.e. hours at most). The primary level of social structure among sperm whale mixed groups appears to be the stable social unit, defined as constant companions who live and move together over periods of years (Whitehead et al. 1991; Christal and Whitehead 2001). Units are not distinguished in the field, but rather recognized through long-term research studies (Christal 1998;



**Figure V.1** Ten sperm whales logging at the surface in the cluster formation.

Christal and Whitehead 2001). The unit has been suggested as being the smallest level of sperm whale social organization containing an average of 13 members each (Christal 1998). Members of units may remain in association over several years or over the course of their lifetime (Whitehead and Arnborn 1987; Crystal 1998; Whitehead and Weilgart 2000). Units in the same area that coalesce over a period of at least days formed groups comprising an average of 23 animals (Whitehead and Kahn 1992). Aggregations are temporary associations of two to several groups that can span areas approximately  $10\text{-}20^2$  km (Whitehead and Kahn 1992).

The inference of social organization among sperm whales within groups was originally based on data collected aboard whaling vessels (Best 1979). In several cases, female members from mixed groups would remain with an injured harpooned member of the group (often small calves), thus leading to their own demise as whalers took advantage of the cohesive nature of these whales (Caldwell and Caldwell 1966). Early tagging studies using mark-recapture techniques provided limited but strong evidence for long-standing associations between individuals within a group (Ohsumi 1971). Sperm whale studies spanning over two decades and utilizing non-lethal research techniques such as photoID, photogrammetry, genetic

sampling and acoustic tracking have provided similar association pattern data for free-ranging whales (Whitehead et al. 1991; Whitehead and Weilgart 2000). These studies suggest three principal functions for sociality in female sperm whales: 1) cooperative foraging, 2) communal care of calves and 3) a collective defence strategy against harassment and predation by other cetaceans such as killer whales (*Orcinus orca*), pilot whales (*Globicephala melas*) and false killer whales (*Pseudorca crassidens*) (Best et al. 1984; Arnborn et al. 1987; Whitehead 1989; Whitehead et al. 1991; Palacios and Mate 1996; Weller et al. 1996; Pitman and Chivers 1999).

#### **V.1.1.2 Bachelor groups**

Males, and occasionally some females, will leave mixed groups and form juvenile or bachelor groups at approximately 4-15 years of age (Gaskin 1970; Best 1979; Whitehead and Arnborn 1987). The data for females found associating with bachelor groups may simply be a by-product of mixed sex social groups being decimated by a recent whaling event, resulting in the few remaining males and females congregating together (Best 1979). Males may then break off from these groups to form aggregations of medium sized bachelors (Best 1979; Rice 1989; Reeves and Whitehead 1997). These aggregations tend to frequent higher latitudes around 55°N and 55°S and appear to lack the degree of close spatial proximity at the surface and strong bonds between individuals that seem so common in female-based mixed sex groups (Best 1979; Whitehead et al. 1991; Whitehead et al. 1992a; Childerhouse et al. 1995; Lettevall et al. 2002). Overall, there is very little detailed information regarding the true underlying genetic makeup of male bachelor groups. Do young male sperm whales maintain familial relationships over time, or are these bachelor groups primarily composed of unrelated whales from not only different natal groups, but perhaps different geographic populations altogether?

#### **V.1.1.3 Solitary males**

Upon nearing sexual maturity at around 27 years of age and lengths of approximately 13.7-15.2 meters, males will often pair up or become solitary whales (Best 1979). At this stage, these large bulls mainly frequent polar waters near latitudes of 70°N and 70°S (Best 1979). Males then return to breed with females that occupy lower latitude

waters (Best 1979; Whitehead 1993). During the breeding season, they appear to apply a “searching bull” strategy with males maintaining temporary associations with females in oestrous over periods of hours to days in an area (Best 1979; Best and Butterworth 1980*b*; Whitehead and Arnborn 1987; Whitehead 1993; Weilgart et al. 1996; Christal 1998; Whitehead and Weilgart 2000). Whitehead and Arnborn (1987) suggest that perhaps due to energetic or other constraints such as increased competition by larger males (Whitehead 1994), younger sexually mature males remain in polar waters throughout the year to feed, thus avoiding the costs associated with breeding on a yearly basis. The reproductive behaviour of male sperm whales may be similar to the “roaming male strategy” described for African elephants (Best 1979; Lee 1991; Weilgart et al. 1996) and various carnivores (Sandell 1989) where males move among relatively philopatric groups of oestrous females, but at a much greater geographic scale than anything previously reported (Lyrholm et al. 1999). Whether the same males return to the same breeding grounds and the same female-based groups year after year, or whether the largest males are the ones achieving the most mating remains unresolved.

### **V.1.2 Understanding Social Structure Through Genetic Techniques**

Previous sperm whale studies incorporating genetic techniques show a significant level of kinship within and between mixed group members, however, not all individuals within a group were related either maternally or paternally (Richard et al. 1996*a*; Christal 1998; Whitehead et al. 1998; Lyrholm et al. 1999). In a study of sperm whales found in three separate groups off Ecuador, whales showed greater genetic relatedness within compared to between groups at five microsatellite DNA markers (Richard et al. 1996*a*). For related members that did not share mtDNA haplotypes, microsatellite profiles revealed probable paternal relatedness (Richard et al. 1996*a*). Christal (1998), Bond (1999) and Lyrholm et al. (1999) all found similar findings with respect to both units and groups. The fact that not all individuals in a group were related either maternally or paternally lends support to Whitehead et al.’s (1991) suggestion that groups are comprised of both constant companions and casual acquaintances.

To better understand the genetic makeup of bachelor groups, levels of relatedness between individuals within two all-male strandings in Scotland were determined by Joanna Bond (1999) using twelve polymorphic microsatellite markers. Bond's (1999) results depict a collection of predominantly unrelated males within each of the stranded groups. However, there were cases for half-siblings within each of the two groups. Due to stranding circumstances, group stranding events may not portray a realistic representation of how free-ranging male groups are structured under normal circumstances in the wild and may lead to misleading conclusions.

Given the previous genetic-based findings that suggest a significant level of relatedness among female dominated mixed sex social groups (Richard et al. 1996a; Christal 1998; Lyrholm et al. 1996, 1999), a better understanding of the relatively unstudied northern Gulf of Mexico (GOM) stock (see Chapter 4) was deemed necessary towards determining the extent of group composition. Although whaling did occur in the GOM from the late 1700's to the early 1900's (Townsend 1935), the total number of whales taken in this area is unknown. In any event, the GOM population of sperm whales is believed to be less affected by large-scale whaling than other populations located in the Pacific and Atlantic Oceans.

## **V.2 QUESTIONS TO ADDRESS**

Population structure based on genetic findings (see Chapter 4) and year-round sightings and re-sightings of individual whales over periods of days to years from numerous distribution and abundance surveys and research cruises in the northern GOM suggests that sperm whales exhibit some degree of philopatry to this geographic area. With the recent expansion of oil and gas exploration and development industries into the relatively untouched deepwater habitats in the GOM, questions relating to group structure exhibited by sperm whales were addressed to provide fundamental information to facilitate effective conservation and management strategies. While Richard et al. (1996a), Christal (1998), Lyrholm et al. (1999) and Bond (1999) have all suggested rather high relatedness levels among members of groups and units, the number of polymorphic microsatellites used in some of these studies may not portray the most accurate representation of relatedness



values. To examine levels of social structure among group and cluster members in the GOM and the North Sea (NSEA), this study analyzed the following:

1. Assess the group structure type (i.e. mixed sex, bachelor or solitary) for sperm whales located in the northern GOM based on molecular sexing methods, estimated sizes for approximate age determination and group size estimates.
2. Determine whether groups and clusters encountered are composed of one or more matriline.
3. Estimate levels of relatedness and degrees of kinship among GOM group members using microsatellite DNA and mtDNA markers. Pairs of individuals within groups were tested for first and second order relations.
4. Establish levels of relatedness and degrees of kinship among two all male stranding events in Scotland using microsatellites DNA and mtDNA markers. Pairs of individuals within groups were tested for first and second order relations.
5. Provide relatedness data for free-ranging whales found in the cluster formation. Pairs of individuals within clusters were tested for first and second order relatedness.
6. Examine group type and relatedness levels for multiple members from six groups of whales tagged with satellite-monitored radio transmitters. Pairs of individuals within groups were tested for first and second order relatedness.



## V.3 RESULTS

### V.3.1 Genetic Composition of GOM Groups

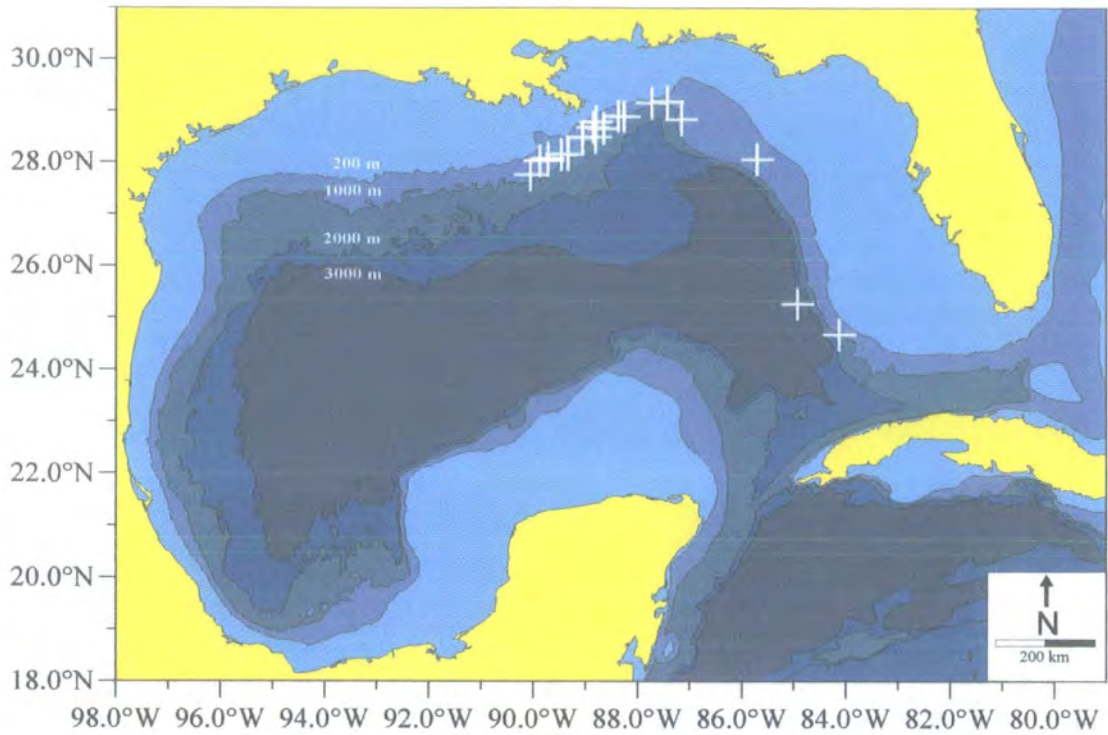
Members from nineteen groups of whales (G0 – G18) were sampled throughout the Mississippi River Canyon, DeSoto Canyon and Dry Tortugas areas during the spring and summer 2000 and 2001 field seasons (Figure V.2). The boundaries of the specified areas are quite arbitrary given the enormous potential for large-scale movement possessed by individual sperm whales (Best 1979; Ivashin 1981; Kasuya and Miyashita 1988; Rice 1989; Dufault and Whitehead 1995). No distinctively ‘large’ whales were found within the study area, giving the impression that physically mature adult males were not present during the study periods.

#### V.3.1.1 Composition of Satellite-Tagged Groups

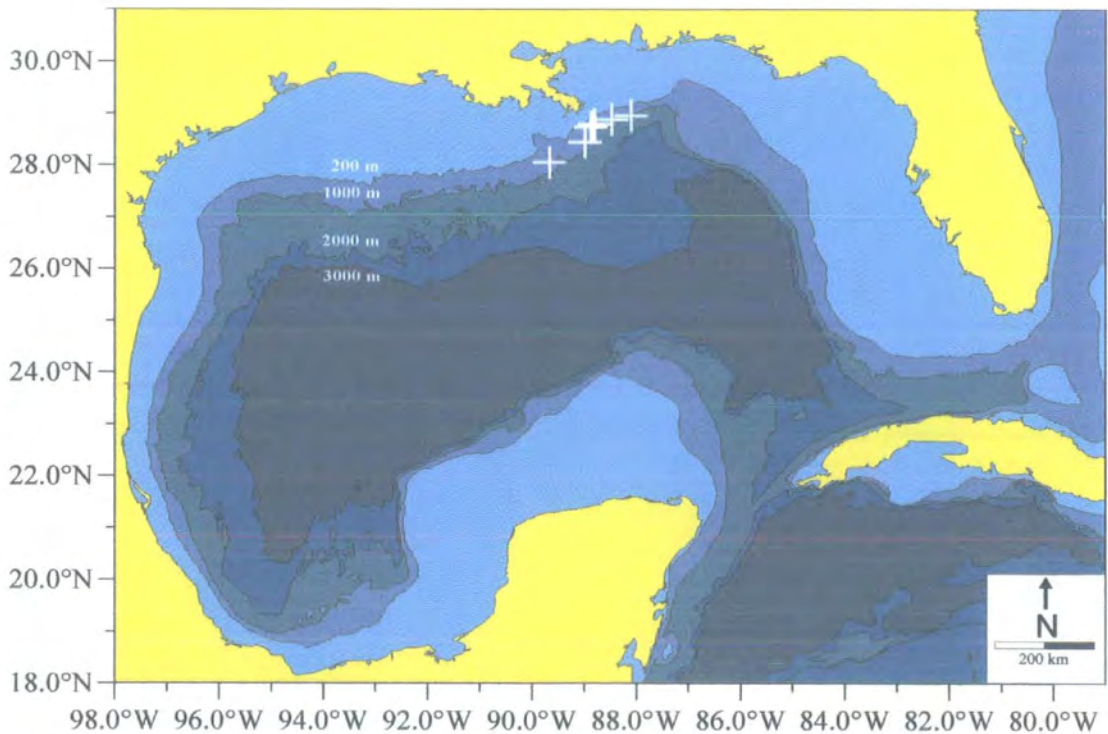
The combination of satellite-monitored tagging and biopsy sampling allows for an in-depth examination of how related and un-related individuals within a group maintain associations through time and space. During the summer of 2002, 20 whales in the northern GOM were biopsy sampled in association with satellite-monitored tag deployments (Figure V.3). Estimated group sizes for six groups (G19 – G24) were determined at the time of tagging. Group size estimates ranged from seven to 18 whales. Genetic relatedness among group members was tested for all sampled whales (including whales with and without satellite-monitored tags). A detailed comparison of genetic relatedness with extremely fine-scale association and movement patterns over days to years are pending Oregon State University’s final analyses of the tag data (Ortega-Ortiz, Engelhaupt and Mate, in prep.).

#### V.3.1.2 Gender composition of groups

The sexual composition of groups G0 to G18 was examined using molecular techniques. The majority (72.3%) of whales sampled in these groups were sexed as females. The males were generally scattered throughout, although there were three cases of all male groups (G6, G7 and G8). These males were thought to be sexually immature based on their estimated sizes. By limiting the dataset to include only groups that have  $\geq 50\%$  of their estimated group size sampled, only groups G0, G1, G3, G4, G5, G6, G7, G8 and G18 were retained. This more conservative approach



**Figure V.2** Locations of 19 groups (G0-G18) sampled in the northern GOM during 2000-2001 are represented by white crosses. Depth contours of 200m, 1000m, 2000m and 3000m are illustrated in shades of blue.



**Figure V.3** Locations of six satellite-monitored tagged groups (G19-G24) sampled in the northern GOM during 2002 are represented by white crosses. Depth contours of 200m, 1000m, 2000m and 3000m are illustrated in shades of blue.

results in 66.6% of the nine groups fitting a mixed sex social group scenario and 33.3% fitting the bachelor group scenario. Of these nine groups, 67.5% of all individuals were females and 32.5% were males. Details for individual groups are provided in Table V.1.

#### **V.3.1.2.1 Gender composition of satellite-tagged groups**

The gender composition of satellite-monitored tag groups was examined to provide an understanding of group type (mixed sex or bachelor) encountered in the northern GOM. Of the twenty whales from groups G19-G24 that were biopsy sampled after tag attachment, 15 (75%) were sexed as females and five (25%) were sexed as males. All whales identified as males were believed to be sexually immature based on estimated sizes. While a broader examination of the gender data continues to provide support for the previous unequal sex ratio results for whales located in the northern GOM, only group G22 contained a sufficient percentage (66.7%) of samples from the estimated group size to be used in further analyses of group composition by gender. Details for individual groups are provided in Table V.2.

#### **V.3.1.3 Pairwise and group relatedness estimates**

All groups were tested as separate entities to provide estimates of relatedness. Estimates of relatedness were determined based on *restricted* and all allele frequency datasets as described in Chapter 2. Incorporating both datasets allowed a means to assess the extent that background alleles may or may not have when calculating R-values. Relatedness values among all nineteen groups ranged from -0.130 to 0.278 for the *restricted* dataset and -0.155 to 0.270 for the all dataset. The mean group relatedness estimate among all 19 groups was 0.067 (std. dev. 0.123) and 0.073 (std. dev. = 0.113) for the *restricted* and all datasets respectively. Pairwise relatedness estimates were obtained for all individual members within their respective group. Estimates of relatedness for all (n=139) pairwise combinations found within each of the 19 groups ranged from -0.284 to 0.675 and -0.285 to 0.666 with an overall mean of 0.063 (std. dev. = 0.193) and 0.054 (std. dev. = 0.192) for the *restricted* and all datasets respectively. Both the *restricted* and all means were not significantly different than an R-value of 0.000 (*restricted* Wilcoxon-Mann-Whitney:  $U = 83.5$ ,  $p$

**Table V.1** Group composition based on estimated size, gender, haplotype and R-value. Italicised R-values were based on the *restricted* set of allele frequencies.

Group #	Group Size (Estimated)	Whales		Male	Haplotype	R-value
		Sampled	Female			
G0*	6	3	2	1	X,X,X	-0.015 -0.009
G1*	6	5	4	1	X,X,X,X,X	0.095 0.070
G2	Unk.	3	3	0	X,X,X	0.278 0.270
G3*	7	7	7	0	X,X,X,X,X,X,X	0.109 0.095
G4*	4	4	3	1	X,X,X,X	-0.013 -0.004
G5*	7	5	4	1	X,X,X,X,X	0.026 0.036
G6*	2	2	0	2	X,X	0.278 0.261
G7*	3	3	0	3	B,Y,C	-0.023 0.037
G8*	6	3	0	3	X,X,X	0.081 0.055
G9	6	2	0	2	B,C	-0.130 -0.155
G10	11	5	4	1	B,B,B,B,B	0.057 0.056
G11	9	4	3	1	B,B,C,C	0.256 0.242
G12	12	2	0	2	X,X	0.194 0.188
G13	12	3	2	1	X,X,Y	0.191 0.201

\*Groups with  $\geq 50\%$  of its members that were sampled.

**Table V.1 (cont.)** Group composition based on estimated size, gender, haplotype and R-value. Italicised R-values were based on the *restricted* set of allele frequencies.

Group #	Group Size (Estimated)	Whales		Female	Male	Haplotype	R-Value
		Sampled					
G14	15	6		5	1	X,X,X,X,X,X	0.012 0.007
G15	25	4		4	0	X,X,Y,Y	0.005 0.021
G16	22	4		4	0	X,X,X,X	0.015 0.005
G17	22	3		3	0	B,B,B	0.069 0.061
G18*	10	8		7	1	Y,Y,Y,X,Y,Y,B,X	0.039 0.024
Total		76		55	21		

\*Groups with  $\geq 50\%$  of its members that were sampled.

$= 0.813_{20,000}$  (Subscript = Monte Carlo resampling size); all Wilcoxon-Mann-Whitney:  $U = 76$ ,  $p = 0.905_{20,000}$ ), which is indicative of non-relatedness among individuals. The distribution of group R-values was centred near zero and suggests individuals within groups are generally unrelated.

Relatedness values for whales found within groups were compared to relatedness levels for whales found between groups to determine whether relatedness is significantly higher within rather than between group members. The mean R-value for all ( $n=2,556$ ) pairwise relatedness comparisons possible between groups G0-G18 was  $-0.013$  (std. dev. =  $0.138$ ) and  $-0.003$  (std. dev. =  $0.137$ ) for the all and *restricted* datasets respectively. A highly significant difference (*restricted* Wilcoxon-Mann-Whitney:  $U = 144863$ ,  $p = 0.000_{20,000}$ ; all Wilcoxon-Mann-Whitney:  $U = 143977$ ,  $p = 0.000_{20,000}$ ) in mean relatedness values was found for whales within vs. between groups with respect to both the *restricted* and all datasets suggesting that whales within a group are more related to one another than whales found between groups.

**Table V.2** Satellite-monitored tagged group composition based on estimated size, gender, haplotype and R-value. Italicised R-values were based on the *restricted* set of allele frequencies.

Group #	Group Size (Estimated)	Whales		Male	Haplotype	R-Value
		Sampled	Female			
G19	8	2	2	0	X,X	-0.000 -0.032
G20	18	5	1	4	A,B,C,C,X	-0.011 0.025
G21	8	2	2	0	X,X	0.027 -0.004
G22*	9	7	7	0	X,X,X,X,X,X,X	-0.014 -0.040
G23	7	2	1	1	Y,X	0.032 0.019
G24	15	2	2	0	X,X	-0.007 -0.042
Total		20	15	5		

\*Group with 50% or more of its members that were sampled.

The following relatedness estimate data describes those groups of whales where  $\geq 50\%$  of the estimated group size was sampled. It is important to note that due to a variety of impeding factors, not all whales within a group were sampled (possible exceptions are groups G3, G4, G6 and G7) and this may affect the group relatedness estimates. Estimated overall group sizes, age-classes and confirmed sexes are provided. Age-classes (immature and adult) are estimated based on visual observations only (opportunistic photogrammetry results taken by other researchers are pending analysis). No young calves were sampled during this study.

Group G0 contained six whales, of which two adult females and one immature male were sampled (Table V.3). The group estimates of relatedness (R-value) were -0.015 (*restricted* data set) and -0.009 (all data set). Pairwise relatedness estimates among individuals ranged from -0.284 to 0.217 (*restricted*) and -0.281 to 0.234 (all). Whale 00070703 was later

resampled in group G3. Two whales from group G0 were re-sampled in group G5 and are listed as sample numbers 00072106 and 00072107 respectively.

**Table V.3** Pairwise relatedness values for Group G0. R-values based on the *restricted* set of allele frequencies are presented in *italics*.

Hap.	Individual	Individual		
		01071901	00072106	00072107
X	01071901	—		
X	00072106	-0.006 <i>-0.000</i>	—	
X	00072107	-0.281 <i>-0.284</i>	0.234 <i>0.217</i>	—

Group G1 contained six whales, of which four adult females and one immature male were sampled (Table V.4). The group estimates of relatedness were *0.095 (restricted)* and 0.070 (all). Pairwise relatedness estimates among group individuals ranged from *-0.166* to *0.540 (restricted)* and -0.173 to 0.525 (all). Whales 00071603 and 00071604 shared an allele at 16 of 16 loci resulting in the substantially high R-value of *0.540* and 0.525.

**Table V.4** Pairwise relatedness values for Group G1. R-values based on the *restricted* set of allele frequencies are presented in *italics*.

Hap.	Individual	Individual				
		00071601	00071602	00071603	00071604	00071605
X	00071601	—				
X	00071602	-0.135 <i>-0.119</i>	—			
X	00071603	-0.021 <i>-0.006</i>	0.039 <i>0.079</i>	—		
X	00071604	-0.173 <i>-0.166</i>	0.058 <i>0.089</i>	0.525 <i>0.540</i>	—	
X	00071605	-0.108 <i>-0.092</i>	0.161 <i>0.199</i>	0.267 <i>0.298</i>	0.119 <i>0.147</i>	—



Group G3 contained seven whales (all of which were sampled), six adult females and one unknown age (length not recorded) female (Table V.5). The group estimates of relatedness were  $0.109$  (*restricted*) and  $0.095$  (all). Pairwise relatedness estimates among group individuals ranged from  $-0.218$  to  $0.675$  (*restricted*) and  $-0.226$  to  $0.666$  (all). One pair of whales (00071904 and 00071905) shared an allele at 16 of 16 loci resulting in the substantially high  $0.675$  and  $0.666$  R-values. Another pair of whales (00071905 and 00071906) also shared an allele at 16 of 16 loci resulting in similar high R-values of  $0.517$  and  $0.516$ .

Table V.5 Pairwise relatedness values for Group G3. R-values based on the *restricted* set of allele frequencies are presented in italics.

Hap.	Individual	Individual						
		00071901	00071904	00071905	00071906	00071907	00071908	00071909
X	00071901	—						
X	00071904	0.127 <i>0.145</i>	—					
X	00071905	0.086 <i>0.099</i>	0.666 <i>0.675</i>	—				
X	00071906	0.083 <i>0.078</i>	0.330 <i>0.337</i>	0.516 <i>0.517</i>	—			
X	00071907	0.255 <i>0.270</i>	-0.080 <i>-0.042</i>	-0.041 <i>-0.013</i>	-0.046 <i>-0.035</i>	—		
X	00071908	0.099 <i>0.125</i>	0.291 <i>0.322</i>	0.082 <i>0.115</i>	0.078 <i>0.094</i>	-0.002 <i>0.042</i>	—	
X	00071909	-0.179 <i>-0.192</i>	-0.094 <i>-0.085</i>	-0.013 <i>-0.014</i>	-0.017 <i>-0.038</i>	-0.226 <i>-0.218</i>	-0.060 <i>-0.046</i>	—

Group G4 contained four whales, three adult females and one immature male (Table V.6). The group estimates of relatedness were *-0.013 (restricted)* and *-0.004 (all)*. Pairwise relatedness estimates among group individuals ranged from *-0.097* to *0.062 (restricted)* and *-0.097* to *0.080 (all)*.

**Table V.6** Pairwise relatedness values for Group G4. R-values based on the *restricted* set of allele frequencies are presented in italics.

Hap.	Individual	Individual			
		00072001	00072002	00072005	00072006
X	00072001	_____			
X	00072002	0.033 <i>0.012</i>	_____		
X	00072005	0.080 <i>0.062</i>	-0.009 <i>-0.019</i>	_____	
X	00072006	-0.089 <i>-0.097</i>	0.051 <i>0.052</i>	-0.097 <i>-0.091</i>	_____

Group G5 contained seven whales, of which three adult females and two immatures (one male and one female) were sampled (Table V.7). The group estimates of relatedness were *0.026 (restricted)* and *0.036 (all)*. Pairwise relatedness estimates among group individuals ranged from *-0.201* to *0.584 (restricted)* and *-0.214* to *0.585 (all)*. Whales 00072105 and 00072106 (an adult female and an immature male) shared an allele at 16 of 16 loci resulting in the substantially high *0.584* and *0.585* R-values. Whales 00072106 and 00072107 were originally sampled in Group G0.

**Table V.7** Pairwise relatedness values for Group G5. R-values based on the *restricted* set of allele frequencies are presented in italics.

Hap.	Individual	Individual				
		00072101	00072102	00072105	00072106	00072107
X	00072101	—				
X	00072102	0.093 <i>0.085</i>	—			
X	00072105	-0.214 <i>-0.222</i>	0.015 <i>0.021</i>	—		
X	00072106	-0.149 <i>-0.168</i>	-0.050 <i>-0.056</i>	0.585 <i>0.584</i>	—	
X	00072107	-0.088 <i>-0.113</i>	-0.185 <i>-0.201</i>	0.148 <i>0.140</i>	0.232 <i>0.217</i>	—

Group 6 contained two males that at the time of sampling were within approximately 5 nautical miles of each other primarily exhibiting foraging dives. These two whales share an allele at 13 of 16 loci. The R-value for this pair/group was *0.278 (restricted)* and 0.261 (all)

Group 7 contained three males (01072701, 00072603 and 00072604) that at the time of sampling were exhibiting foraging dives within approximately 5 nautical miles of each other (Table V.8). All three whales possessed different haplotypes suggesting no relatedness via a common maternal line. The group estimates of relatedness were *-0.023 (restricted)* and -0.037 (all). Pairwise relatedness estimates among group individuals ranged from *-0.127 to 0.078 (restricted)* and -0.144 to 0.068 (all).

**Table V.8** Pairwise relatedness values for Group G7. R-values based on the *restricted* set of allele frequencies are presented in italics.

Hap.	Individual	Individual		
		00072601	00072603	00072604
B	01072701	—		
Y	00072603	-0.030 <i>-0.016</i>	—	
C	00072604	-0.144 <i>-0.127</i>	0.068 <i>0.078</i>	—

Group G8 contained three males (01072801, 01072802, and 01072803) that at the time prior to and after sampling were exhibiting foraging dives within 5 nautical miles of each other (Table V.9). In the case of individuals 01072801 and 01072802, these whales were sampled as they logged side-by-side (within 5 meters of each other) in the cluster formation. The group estimates of relatedness were *0.081 (restricted)* and 0.055 (all). Pairwise relatedness estimates among group individuals ranged from -0.259 to *0.463 (restricted)* and -0.285 to 0.439 (all). Individuals 01072801 and 01072802 shared an allele at 15 of 16 loci, the same mtDNA haplotype (haplotype 'X') and R-values of *0.463* and 0.439. Given haplotype X's uniqueness to the GOM, it is suggested this pair may be related at either the level of full or half-siblings.

**Table V.9** Pairwise relatedness values for Group G8. R-values based on the *restricted* set of allele frequencies are presented in italics.

Hap.	Individual	Individual		
		01072801	01072802	01072803
X	01072801	—		
X	01072802	0.439 <i>0.463</i>	—	
X	01072803	0.059 <i>0.077</i>	-0.285 <i>-0.259</i>	—

Group G18 contained ten whales, of which three adult females, three immature females, one immature male and one unknown (length not recorded) female were sampled (Table V.10). Interestingly, three separate haplotypes were represented in this group. The group estimates of relatedness were *0.039 (restricted)* and 0.024 (all). Pairwise relatedness estimates among group individuals ranged from -0.202 to *0.591 (restricted)* and -0.222 to 0.580 (all). Whales 01081802 and 01081806 shared an allele at 16 of 16 loci resulting in the substantially high *0.591* and 0.580 R-values. Two whales (01081507 and 01081505) were previously sampled in group G12.

**Table V.10** Pairwise relatedness values for Group G18. R-values based on the *restricted* set of allele frequencies are presented in italics.

Hap.	Individual	Individual							
		01081801	01081802	01081507	01081505	01081805	01081806	01081807	01081808
Y	01081801	—							
Y	01081802	0.024 <i>0.030</i>	—						
Y	01081507	0.038 <i>0.033</i>	0.153 <i>0.166</i>	—					
X	01081505	-0.165 <i>-0.167</i>	0.001 <i>0.021</i>	0.017 <i>0.024</i>	—				
Y	01081805	0.059 <i>0.063</i>	0.074 <i>0.096</i>	-0.050 <i>-0.037</i>	-0.007 <i>0.011</i>	—			
Y	01081806	0.051 <i>0.057</i>	0.580 <i>0.591</i>	-0.106 <i>-0.089</i>	-0.065 <i>-0.044</i>	0.053 <i>0.075</i>	—		
B	01081807	-0.095 <i>-0.083</i>	0.005 <i>0.035</i>	-0.071 <i>-0.050</i>	0.111 <i>0.132</i>	0.081 <i>0.106</i>	-0.011 <i>0.019</i>	—	
X	01081808	-0.032 <i>-0.036</i>	-0.222 <i>-0.202</i>	0.097 <i>0.102</i>	0.259 <i>0.266</i>	-0.025 <i>-0.010</i>	-0.081 <i>-0.063</i>	-0.001 <i>0.020</i>	—

#### V.3.1.3.1 *Pairwise and group relatedness estimates – satellite-tagged groups*

Levels of relatedness from whales within the six satellite-monitored tagging groups were determined based on 12 polymorphic microsatellite loci. No groups were sampled in their entirety. All groups were tested for relatedness within and between groups. Between group relatedness ranged from  $-0.014$  to  $0.032$  for the restricted dataset and  $-0.042$  to  $0.019$  for the all dataset with a mean of  $0.005$  (std. dev. =  $0.020$ ) and  $-0.020$  (std. dev. =  $0.024$ ) for the *restricted* and all datasets respectively. Estimates of relatedness for 35 pairwise combinations within each of the six groups ranged from  $-0.328$  to  $0.390$  and  $-0.364$  to  $0.353$  with an overall mean of  $-0.011$  (std. dev. =  $0.141$ ) and  $-0.033$  (std. dev. =  $0.144$ ) for the *restricted* and all datasets respectively. These means were not statistically different than an R-value of  $0.000$  (*restricted* Wilcoxon-Mann-Whitney:  $U = 17.5$ ,  $p = 1.000_{20,000}$ ; all Wilcoxon-Mann-Whitney:  $U = 12$ ,  $p = 0.723_{20,000}$ ), which is indicative of non-relatedness among individuals. The distribution of all group R-values was centred near zero and suggests that the majority of individuals within groups were not highly related.

No significant difference (*restricted* Wilcoxon-Mann-Whitney:  $U = 2588$ ,  $p = 0.765_{20,000}$ ; all Wilcoxon-Mann-Whitney:  $U = 2595$ ,  $p = 0.777_{20,000}$ ) in relatedness levels was detected when whales found within S-tagged groups were compared to whales found between S-tagged groups with respect to both the all and *restricted* dataset.

The following relatedness estimate data describes only those groups of whales where  $> 2$  members of the group were both sampled and tagged. Age-classes (immature and adult) were estimated based on visual observations only. No young calves were sampled or tagged during this study.

Group G20 contained approximately 18 whales, of which four immature males and one adult female were sampled (Table V.11). Two individual males (02070103 and 02070104) within the group shared the same haplotype (C), while the other three whales carried the A, B and X haplotypes. The group estimates of relatedness were *-0.011 (restricted)* and *-0.025 (all)*. Pairwise relatedness estimates among group individuals ranged from *-0.242* to *0.110 (restricted)* and *-0.279* to *0.110 (all)*. The female (02070105) shared neither a haplotype nor a strong relatedness value with any of the young males.

**Table V.11** Pairwise relatedness values for Group G20. R-values based on the *restricted* set of allele frequencies are presented in italics.

Hap.	Individual	Individual				
		02070101	02070102	02070103	02070104	02070105
A	02070101	_____				
X	02070102	0.070 <i>0.094</i>	_____			
C	02070103	-0.153 <i>-0.127</i>	0.072 <i>0.088</i>	_____		
C	02070104	-0.279 <i>-0.242</i>	-0.122 <i>-0.097</i>	0.031 <i>0.049</i>	_____	
B	02070105	-0.017 <i>-0.012</i>	0.110 <i>0.111</i>	0.043 <i>0.041</i>	-0.052 <i>-0.052</i>	_____

Group G22 contained approximately eleven whales, of which seven adult females were sampled (Table V.12). All whales within the group shared the same 'X' haplotype. The group estimates of relatedness were *-0.014 (restricted)* and *-0.040 (all)*. Pairwise relatedness estimates among group individuals ranged from *-0.327* to *0.390 (restricted)* and *-0.364* to *0.353 (all)*. Interestingly, four whales (00071904, 00071907, 00071908 and 00071909) were previously sampled two years earlier in July 2000 when they were members of group G3 (see Group G3 for pairwise results based on sixteen microsatellites).

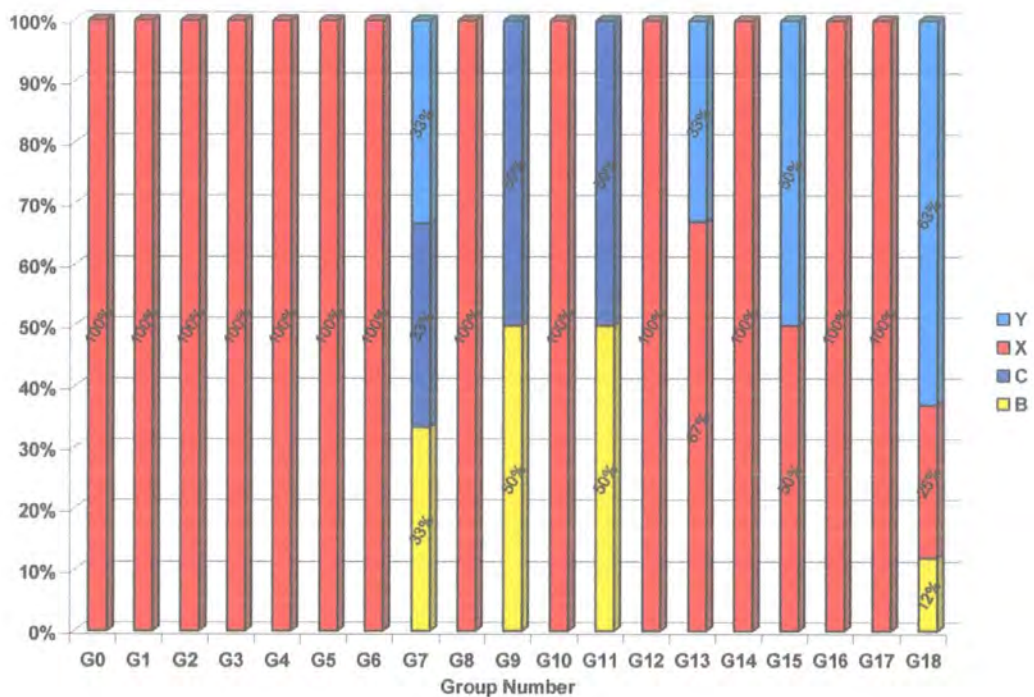


**Table V.12** Pairwise relatedness values for Group G22. R-values based on the *restricted* set of allele frequencies are presented in italics.

Hap.	Individual	Individual						
		00071909	00071907	02070303	02070304	00071904	02070202	00071908
X	00071909	—						
X	00071907	-0.196 <i>-0.180</i>	—					
X	02070303	0.112 <i>0.111</i>	-0.364 <i>-0.328</i>	—				
X	02070304	-0.041 <i>-0.043</i>	0.347 <i>0.362</i>	-0.232 <i>-0.220</i>	—			
X	00071904	-0.162 <i>-0.134</i>	-0.144 <i>-0.087</i>	-0.015 <i>0.021</i>	-0.096 <i>-0.062</i>	—		
X	02070202	0.018 <i>0.013</i>	-0.128 <i>-0.101</i>	0.057 <i>0.066</i>	-0.080 <i>-0.072</i>	-0.050 <i>-0.015</i>	—	
X	00071908	-0.008 <i>0.013</i>	0.009 <i>0.059</i>	-0.021 <i>0.014</i>	-0.157 <i>-0.123</i>	0.353 <i>0.390</i>	-0.004 <i>0.027</i>	—

### V.3.1.4 Matriline composition within groups

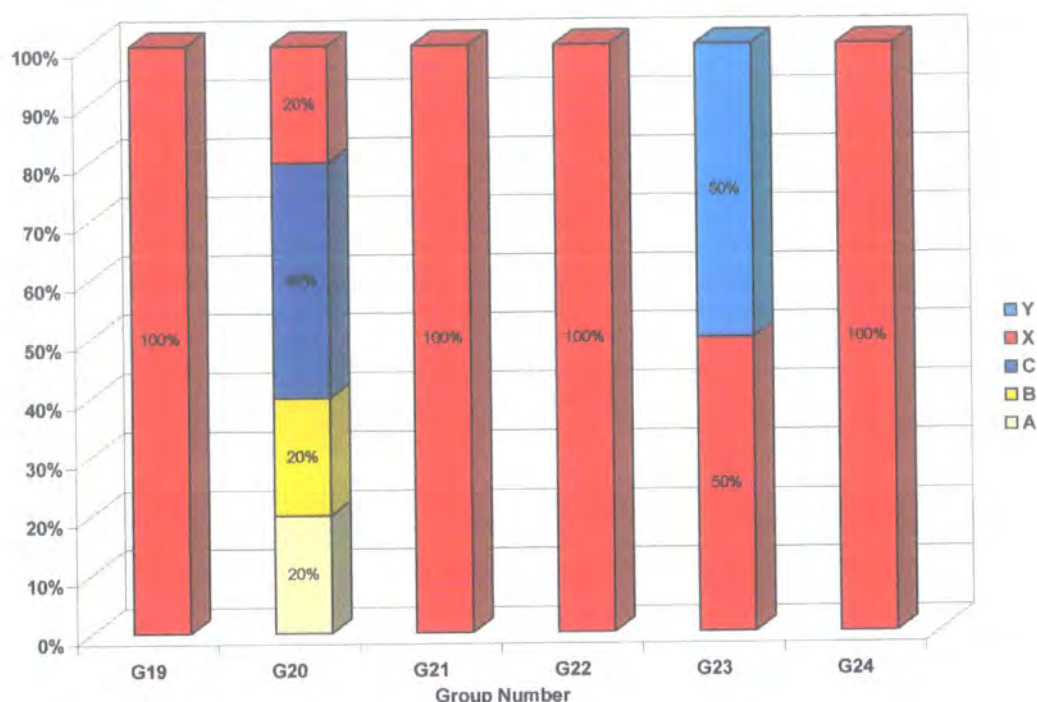
All sperm whales sampled in the northern GOM contained one of five haplotypes (A, B, C, X and Y). To date, no additional lineages have been found in samples throughout the GOM. When combined with results on a global scale, haplotypes X and Y appear to be unique to the GOM (see Chapter 4). Figure V.4 represents the percentage of sampled members haplotypes (B, C, X and Y) within each of the GOM groups.



**Figure V.4** Percentage of haplotypes B, C, X and Y within the 19 GOM groups.

#### V.3.1.4.1 Matriline composition within s-tagged groups

Sperm whales sampled in the northern GOM during satellite-monitored tagging operations contained one of five haplotypes (A, B, C, X and Y). Figure V.5 represents the haplotype percentage for members of each of the satellite-monitored tag groups.



**Figure V.5** Percentage of haplotypes A, B, C, X and Y within the six satellite-monitored tagged groups.

#### **V.3.1.5 Putative highly related pairs within groups**

Highly related pairs (e.g. mother-offspring, full-siblings, half-siblings and grandparent-grandchild) within groups were identified via an examination of R-values determined with Relatedness 5.0.2 and Kinship 1.3.1 software. Alleles shared across 16 polymorphic loci between possible pairs were examined by eye. Out of 76 individuals comprising 19 groups, only nineteen potential highly related pairs were identified within 12 of the 19 groups (Table V.13). Of the nine groups with  $\geq 50\%$  of their members sampled, six groups contained highly related pairs within their respective group. Degrees of relatedness between relations were tested for three scenarios – parent-offspring, full-siblings and half-siblings using the likelihood method previously described. Due to the lack of additional long-term behavioural information, relatedness values could not provide further clarification about which relationship between individuals was the correct one. Certain relationships that tested positive for mother-offspring or full-siblings pairs were discarded after

Table V.13 Potential relatives within groups. Italicised relationships tested (*YES/NO*) were based on the *restricted* set of allele frequencies.

First Order Relatives	Sex	Hap	Group	# Seen / # Sampled	Loci With Shared Alleles			Relationship Tested		
					2 Alleles	1 Allele	0 Alleles	Parent-Offspring	Full-Sibs	Half-Sibs
										Mat. / Pat.
00071603 / 00071604	F/F	X/X	G1*	6 / 5	3	13	0	YES <sup>++</sup>	YES <sup>++</sup>	YES <sup>++</sup> / YES <sup>++</sup>
								<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup> / YES<sup>++</sup></i>
00071701 / 00071702	F/F	X/X	G2	Unk. / 3	6	10	0	YES <sup>++</sup>	YES <sup>++</sup>	YES <sup>++</sup> / YES <sup>++</sup>
								<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup> / YES<sup>++</sup></i>
00071904 / 00071905	F/F	X/X	G3*	7 / 7	7	9	0	YES <sup>++</sup>	YES <sup>++</sup>	YES <sup>++</sup> / YES <sup>++</sup>
								<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup> / YES<sup>++</sup></i>
00071905 / 00071906	F/F	X/X	G3*	7 / 7	3	13	0	YES <sup>++</sup>	YES <sup>++</sup>	YES <sup>++</sup> / YES <sup>++</sup>
								<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup> / YES<sup>++</sup></i>
00071904 / 00071906	F/F	X/X	G3*	7 / 7	3	11	2	NO	NO	NO / NO
								<i>NO</i>	<i>NO</i>	<i>NO / YES<sup>+</sup></i>
00071904 / 00071908	F/F	X/X	G3*	7 / 7	3	9	4	NO	NO	NO / NO
								<i>NO</i>	<i>NO</i>	<i>YES<sup>+</sup> / YES<sup>+</sup></i>
00072105 / 00072106	F/M	X/X	G5*	7 / 5	6	10	0	YES <sup>++</sup>	YES <sup>++</sup>	YES <sup>++</sup> / YES <sup>++</sup>
								<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup> / YES<sup>++</sup></i>
00072401 / 00072402	M/M	B/B	G6*	2 / 2	1	12	3	NO	YES <sup>+</sup>	YES <sup>++</sup> / YES <sup>++</sup>
								<i>NO</i>	<i>YES<sup>+</sup></i>	<i>YES<sup>++</sup> / YES<sup>++</sup></i>

\*Group with  $\geq 50\%$  of its members that were sampled. <sup>+</sup>Significance value ( $p < 0.01$ ), <sup>++</sup>Significance value ( $p < 0.001$ )

**Table V.13 (continued)** Potential relatives within groups. *Italicised relationships tested (YES/NO) were based on the restricted set of allele frequencies.*

First Order Relatives	Sex	Hap	Group	# Seen / # Sampled	Loci With Shared Alleles			Relationship Tested		
					2 Alleles	1 Allele	0 Alleles	Parent-Offspring	Full-Sibs	Half-Sibs
										Mat. / Pat.
01072801 / 01072802	M/M	X/X	G8*	6 / 3	4	11	1	NO	YES <sup>++</sup>	YES <sup>++</sup> / YES <sup>++</sup>
								<i>NO</i>	<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup> / YES<sup>++</sup></i>
01032601 / 01032602	F/F	B/B	G11	9 / 4	6	10	0	YES <sup>++</sup>	YES <sup>++</sup>	YES <sup>++</sup> / YES <sup>++</sup>
								<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup> / YES<sup>++</sup></i>
01032603 / 01032607	F/M	C/C	G11	9 / 4	5	11	0	YES <sup>++</sup>	YES <sup>++</sup>	YES <sup>++</sup> / YES <sup>++</sup>
								<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup> / YES<sup>++</sup></i>
01081301 / 01081302	F/F	X/X	G13	12 / 3	4	12	0	YES <sup>++</sup>	YES <sup>++</sup>	YES <sup>++</sup> / YES <sup>++</sup>
								<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup> / YES<sup>++</sup></i>
01081401 / 01081404	F/M	X/X	G14	15 / 6	4	8	4	NO	YES <sup>+</sup>	YES <sup>+</sup> / YES <sup>+</sup>
								<i>NO</i>	<i>YES<sup>+</sup></i>	<i>YES<sup>+</sup> / YES<sup>+</sup></i>
01081406 / 01081407	F/F	X/X	G14	15 / 6	2	13	1	NO	YES <sup>+</sup>	YES <sup>+</sup> / YES <sup>+</sup>
								<i>NO</i>	<i>YES<sup>+</sup></i>	<i>YES<sup>+</sup> / YES<sup>+</sup></i>
01081505 / 01081506	F/F	X/X	G15	25 / 4	4	11	1	NO	YES <sup>+</sup>	YES <sup>+</sup> / YES <sup>+</sup>
								<i>NO</i>	<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup> / YES<sup>++</sup></i>

\*Group with  $\geq 50\%$  of its members that were sampled. <sup>+</sup>Significance value ( $p < 0.01$ ), <sup>++</sup>Significance value ( $p < 0.001$ )

**Table V.13 (continued)** Potential relatives within groups. Italicised relationships tested (*YES/NO*) were based on the *restricted* set of allele frequencies.

First Order Relatives	Sex	Hap	Group	# Seen / # Sampled	Loci With Shared Alleles			Relationship Tested		
					2 Alleles	1 Allele	0 Alleles	Parent-Offspring	Full-Sibs	Half-Sibs
										Mat. / Pat.
01081701 / 01081702	F/F	B/B	G17	22 / 3	3	11	2	NO	YES <sup>++</sup>	YES <sup>++</sup> / YES <sup>++</sup>
								<i>NO</i>	<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup> / YES<sup>++</sup></i>
01081802 / 01081806	F/F	Y/Y	G18*	10 / 8	7	9	0	YES <sup>++</sup>	YES <sup>++</sup>	YES <sup>++</sup> / YES <sup>++</sup>
								<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup> / YES<sup>++</sup></i>
01081505 / 01081808	F/F	X/X	G18*	10 / 8	5	9	2	NO	YES <sup>+</sup>	NO / NO
								<i>NO</i>	<i>YES<sup>+</sup></i>	<i>NO / NO</i>

\*Group with  $\geq 50\%$  of its members that were sampled. <sup>+</sup>Significance value ( $p < 0.01$ ), <sup>++</sup>Significance value ( $p < 0.001$ )

comparing mtDNA haplotypes. For example, the female pair 01032602 / 01032603 that passes for a full-sibling pair ( $p < 0.01$ ) cannot be correct as they come from two different maternal lineages ('B' and 'C' respectively). However, the likelihood of this pair being half-sisters that are related via paternal lines remains plausible ( $p < 0.001$ ).

Nine pairs show R-values that represent parent-offspring, full-siblings or half-siblings based on log-likelihood testing. The two female pairs from group G3 (00071904 / 00071905 and 00071905 / 00071906) share individual 00071905 within each pair. Given the degree of allele sharing between paired individuals (at least one allele at each loci) and the lack of a substantially high R-value between 00071904 and 00071906; a plausible scenario is that 00071905 is the mother of both 00071904 and 00071906 and that these latter two are related as half-siblings ( $p < 0.05$ ).

Interestingly, two of the all male groups (G6 and G8) contained potential full-sibling pairs. None of the members of the two sampled pairs were deemed large enough to be considered sexually mature. Although the 00072401 / 00072402 pair did not share an allele at 3 of the 16 loci, according to Mendelian genetics they could still be related at the level of full-siblings. The second pair (01072801 / 01072802) not only shared an allele at 15 of 16 loci, but carried the same 'X' haplotype. The lack of sharing only one allele at one of 16 polymorphic loci could still suggest that these whales are full-siblings and share a common father and mother. However, given the similar estimated length observed in the field, gestation period and growth curve data presented by Best (1979), the most likely scenario would be that these two whales are related at the half-sibling level (sharing a common mother or father – either is possible in this case).

#### **V.3.1.5.1 Potential relative pairs within satellite-tagged groups**

Following an examination of R-values determined with Kinship 1.3.1 software, two highly related pairs were identified within only one of the six groups containing whales tagged with satellite-monitored transmitters (Table V.14). Group G22 was thought to have the majority (66%) of its members sampled. Neither of the pairs tested positive for parent-offspring relationships. Full-sib relationship was positive, but only for the *restricted* dataset. A half-sibling relationship via maternal or paternal lines was likely for both pairs.

**Table V.14** Potential highly related whales within satellite-tagged groups. Italicised relationships tested (*YES/NO*) were based on the *restricted* set of allele frequencies.

First Order Relatives	Sex	Hap	Group	# Seen / # Sampled	Loci With Shared Alleles			Relationship Tested		
					2 Alleles	1 Allele	0 Alleles	Parent-Offspring	Full-Sibs	Half-Sibs Mat. / Pat.
00071907 / 02070304	F/F	X/X	22 <sup>*</sup>	9/7	3	6	3	NO	NO	YES <sup>+</sup> / YES <sup>+</sup>
								<i>NO</i>	<i>YES<sup>+</sup></i>	<i>YES<sup>+</sup> / YES<sup>+</sup></i>
00071904 / 00071908	F/F	X/X	22 <sup>*</sup>	9/7	3	7	2	NO	NO	NO / YES <sup>+</sup>
								<i>NO</i>	<i>YES<sup>+</sup></i>	<i>YES<sup>+</sup> / YES<sup>+</sup></i>

\*Group with 50% or more of its members that were sampled.

<sup>+</sup>Significance value ( $p < 0.01$ ), <sup>++</sup>Significance value ( $p < 0.001$ )



### **V.3.2 Genetic Composition of Clusters**

Clusters vary from groups mainly with respect to the behaviour exhibited and the distance separating whales at the surface. Two individuals from each of six clusters (A, B, C, E, F and G), three from cluster D and four from cluster I were sampled using biopsy and sloughed skin collection methods. All clusters described here were imbedded within groups and therefore share the same locations as six of the 19 groups seen in Figure V.2. Additional information such as cluster sizes, number of samples/cluster, gender, haplotype and relatedness values are provided in Table V.15. Given the cluster size range of two to ten whales, it is clear that not all clusters were sampled in their entirety and caution should be taken when interpreting the results. However, clusters C, F and G were sampled in full.

#### **V.3.2.1 Genetic Composition of Satellite-Monitored Tagging Clusters**

Satellite-monitored tagged whales with accompanying genetic material were collected from four clusters (J, K, L and M). All S-tag clusters described here were embedded within S-tag groups and therefore share two of the six locations seen in Figure V.3. Additional information such as cluster sizes, number of samples/cluster, gender, haplotype and relatedness values are provided in Table V.16. Only clusters K and L contained  $\geq 50\%$  of the cluster size at the time of sampling.

**Table V.15** Cluster composition based on whales sampled within an estimated cluster size, gender, haplotypes and R-values. Italicised R-values were based on the *restricted* set of allele frequencies.

Cluster		Whales				
Cluster	Size	Sampled	Female	Male	Haplotypes	R-value
A*	4	2	1	1	X,X	-0.119 -0.135
B*	4	2	2	0	X,X	0.147 0.119
C*	2	2	2	0	X,X	0.566 0.560
D*	5	3	3	0	X,X,X	-0.074 -0.098
E*	3	2	2	0	X,X	0.085 0.093
F*	2	2	1	1	X,X	0.585 0.584
G*	2	2	0	2	X,X	0.463 0.439
I	10	4	4	0	X,X,Y,Y	-0.005 -0.021
Total		19	15	4		

\*Cluster with  $\geq 50\%$  or more of its members that were sampled.

**Table V.16** Satellite-monitored tagged cluster composition based on estimated cluster size, gender, haplotype and R-values. Italicised R-values were based on the *restricted* set of allele frequencies.

Cluster		Whales				
Cluster	Size	Sampled	Female	Male	Haplotypes	R-value
J <sup>†</sup>	9	3	0	3	A,C,X	0.020 -0.001
K* <sup>†</sup>	3	2	1	1	C,B	-0.052 -0.052
L* <sup>^</sup>	2	2	1	1	X,X	0.014 -0.021
M <sup>^</sup>	7	2	2	0	X,X	0.027 -0.004
Total		9	4	5		

\*Cluster with 50% or more of its members that were sampled. <sup>†</sup> denotes a cluster embedded within group G20. <sup>^</sup> denotes a cluster embedded within group G22.

### V.3.2.2 Gender composition of clusters

Fifteen females (78.9%) and four males (21.1%) sampled from eight free-ranging clusters in the northern GOM were confirmed using molecular sexing methods.

Females within a given cluster varied from juveniles to adults (based on approximate sizes estimated from small boat personnel), while all males within a cluster were considered sexually immature based on estimated sizes. If we limit our dataset to incorporate only clusters that have  $\geq 50\%$  or more of their estimated cluster size sampled, then we retain clusters A through G. Only three of these seven clusters were sampled in their entirety. This more conservative approach results in four clusters comprised of females only, two clusters including males and females and only one containing all males. Of these seven clusters, 73.3% of all individuals sampled while in a cluster formation were females and 26.6% were males. Although it would appear that clusters in the northern GOM are primarily composed of females and/or female-young male combinations, this may simply be a result of the full GOM sample set being dominated by females.

### V.3.2.2.1 Gender composition of satellite-tagged clusters

Four females (44.4%) and five males (55.6%) were sampled from four free-ranging clusters in the northern GOM during 2002. Both females and males found within a given cluster ranged in size (based on approximate sizes estimated from small boat personnel) and thus varied in estimated age-class. If we limit our dataset to incorporate only clusters that have  $\geq 50\%$  of their estimated cluster size sampled, then we retain clusters K and L only. Cluster L contained two whales, both of which were sampled. Of these two clusters, 50% of all individuals sampled while in a cluster formation were females and 50% were young/immature males. This more conservative approach results in 100% mixed sex makeup for satellite-tagged clusters.

### V.3.2.3 Pairwise and cluster relatedness estimates

All clusters were tested as separate entities to provide estimates of relatedness. Individual cluster relatedness values over all eight clusters ranged from  $-0.119$  to  $0.585$  for the *restricted* dataset and  $-0.135$  to  $0.584$  for the all dataset. The mean cluster relatedness estimate over all eight clusters was  $0.206$  (std. dev. =  $0.289$ ) and  $0.193$  (std. dev. =  $0.293$ ) for the *restricted* and all datasets respectively. Pairwise relatedness estimates were obtained for all individual members within their respective cluster. Estimates of relatedness for all 15 pairwise combinations within each of the nine clusters ranged from  $-0.224$  to  $0.585$  and  $-0.241$  to  $0.584$  with an overall mean of  $0.100$  (std. dev. =  $0.263$ ) and  $0.084$  (std. dev. =  $0.266$ ) for the *restricted* and all datasets respectively. These means were not statistically different than an R-value of  $0.000$  (*restricted* Wilcoxon-Mann-Whitney:  $U = 8$ ,  $p = 1.000_{20,000}$ ; all Wilcoxon-Mann-Whitney:  $U = 7$ ,  $p = 1.000_{20,000}$ ), which is indicative of non-relatedness among individuals. The distribution of all cluster R-values was centred near zero, which suggests that individuals within clusters were not highly related.

#### V.3.2.3.1 *Pairwise and cluster relatedness estimates – satellite-tagged clusters*

All clusters were tested separately and together to provide estimates of relatedness. The mean cluster relatedness estimate over all four S-tagged clusters was 0.002 (std. dev. = 0.037) and -0.020 (std. dev. = 0.023) for the *restricted* and all datasets respectively. Pairwise relatedness estimates were obtained for all individual members within their respective cluster. Estimates of relatedness for all six pairwise combinations within each of the four clusters ranged from -0.127 to 0.094 and -0.153 to 0.072 with an overall mean of -0.002 (std. dev. = 0.085) and -0.015 (std. dev. = 0.084) for the *restricted* and all datasets respectively. These means were not statistically different than an R-value of 0.000 (*restricted* Wilcoxon-Mann-Whitney:  $U = 3, p = 1.000_{20,000}$ ; all Wilcoxon-Mann-Whitney:  $U = 2, p = 0.858_{20,000}$ ), which is indicative of non-relatedness among individuals. The distribution of all group R-values was centred near zero and suggests that individuals within clusters were not highly related.

#### V.3.2.4 *Potential relative pairs within clusters*

First order relative pairs within clusters were identified upon an examination of R-values determined with Relatedness 5.0.2 and Kinship 1.3.1 software. Out of 19 individuals comprising eight clusters, only three potential highly related pairs were identified within three separate clusters (C, F and G). One pair was from the male only cluster G. Potential parent-offspring relationships were only possible within clusters C and F as cluster G was composed of two young males. Both pairs in clusters C and F shared alleles at 16 of 16 loci and provided significant likelihood results for each relationship tested. The male sampled in cluster F was deemed immature based on estimated size. Table V.17 provides a summary of likely relative pairs. Cluster G could be composed of either full or half-sibs. The two males sampled in cluster G were believed to be immature based on estimated size.

**Table V.17** Potential highly related whales within clusters. Italicised relationships tested (*YES/NO*) were based on the *restricted* set of allele frequencies.

First Order Relatives	Sex	Hap	Cluster	# Seen / # Sampled	Loci With Shared Alleles			Relationship Tested		
					2 Alleles	1 Allele	0 Alleles	Parent-Offspring	Full-Sibs	Half-Sibs
										Mat. / Pat.
00071701 / 00071702	F/F	X/X	C*	2/2	6	10	0	YES <sup>++</sup>	YES <sup>++</sup>	YES <sup>++</sup> / YES <sup>++</sup>
								<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup> / YES<sup>++</sup></i>
00072105 / 00072106	F/M	X/X	F*	2/2	6	10	0	YES <sup>++</sup>	YES <sup>++</sup>	YES <sup>++</sup> / YES <sup>++</sup>
								<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup> / YES<sup>++</sup></i>
01072801 / 01072802	M/M	X/X	G*	2/2	4	11	1	NO	YES <sup>++</sup>	YES <sup>++</sup> / YES <sup>++</sup>
								NO	<i>YES<sup>++</sup></i>	<i>YES<sup>++</sup> / YES<sup>++</sup></i>

\*Group with 50% or more of its members that were sampled. <sup>+</sup>Significance value ( $p < 0.01$ ), <sup>++</sup>Significance value ( $p < 0.001$ )

#### **V.3.2.4.1 Potential relative pairs within satellite-tagged clusters**

No potential first order relative pairs were identified within any of the four clusters containing whales that were tagged with satellite-monitored tags.

#### **V.3.2.5 Cluster vs. group comparisons**

The mean relatedness value for whales found within clusters was compared to the mean relatedness level for whales found within groups to determine whether relatedness is higher within clusters rather than groups. No significant difference (*restricted* Wilcoxon-Mann-Whitney:  $U = 1030, p = 0.942_{20,000}$ ; all Wilcoxon-Mann-Whitney:  $U = 1051, p = 0.959_{20,000}$ ) was found when clusters A-I were compared with the mean relatedness values of whales found within groups G0-G18. This suggests that clusters and groups share a low level of relatedness among members.

##### **V.3.2.5.1 Satellite-tagged clusters vs. satellite-tagged groups**

The mean relatedness value for whales found within S-tagged clusters was compared to the mean relatedness level for whales found within S-tagged groups to determine whether relatedness is higher within S-tagged clusters rather than within and between S-tagged groups. The mean R-value for all ( $N = 6$ ) pairwise relatedness comparisons found within each of the four clusters was  $-0.002$  (*std. dev.* =  $0.085$ ) and  $-0.015$  (*std. dev.* =  $0.084$ ) for the *restricted* and all datasets respectively. No significant difference (*restricted* Wilcoxon-Mann-Whitney:  $U = 97.5, p = 0.794_{20,000}$ ; all Wilcoxon-Mann-Whitney:  $U = 91.5, p = 0.633_{20,000}$ ) was found when S-tagged clusters were compared with the mean relatedness values for whales found within S-tagged groups.

#### **V.3.3 Genetic Composition of North Sea Strandings**

In addition to the samples collected from the northern GOM area, this analysis incorporated young male sperm whales found stranded together in groups along the Scottish coast in 1994 and 1996 (Figure IV.8, Chapter 4). Group NS1 included eleven whales stranded along the Orkney coast. Group NS2 was comprised of six whales stranded on the Grampian coast. Size estimates ranging from 12 to 15 meters suggest that these whales were not physically mature at the time of stranding and

they may have comprised a bachelor group (Best 1979; Bond 1999; Childerhouse et al. 1995; Lettevall et al. 2002; Rice 1989).

#### V.3.3.1 *Pairwise and group relatedness estimates*

There appears to be some degree of genetic similarity between males found in the NSEA and whales found in the northern GOM (see Chapter 4). In an attempt to eliminate potential biases when performing pairwise and group relatedness calculations for the NSEA strandings, R-value estimates were calculated using the GOM *restricted* and all sets of allele frequencies. Both groups were tested separately to provide estimates of relatedness. The mean group relatedness estimate over both groups was 0.037 (*std. dev.* = 0.059) and 0.032 (*std. dev.* = 0.053) for the *restricted* and all datasets respectively. Pairwise relatedness estimates were obtained for all individual members within their respective group. Estimates of relatedness for all 70 pairwise combinations found within each of the two groups ranged from -0.242 to 0.396 and -0.259 to 0.393 with an overall mean of 0.058 (*std. dev.* = 0.139) and 0.047 (*std. dev.* = 0.142) for the *restricted* and all datasets respectively. These means were not statistically different than an R-value of 0.000 (*restricted* Wilcoxon-Mann-Whitney:  $U = 41$ ,  $p = 0.844_{20,000}$ ; all Wilcoxon-Mann-Whitney:  $U = 40$ ,  $p = 0.872_{20,000}$ ) and suggest that males found within groups are primarily unrelated.

Group NSI contained a total of eleven males. The majority of whales shared the third most common mtDNA haplotype 'C', although haplotype 'A' and 'B' were present in the group (Table V.18). The group estimates of relatedness were 0.079 (*restricted*) and 0.069 (all) suggesting overall low relatedness. Pairwise relatedness estimates among group individuals ranged from -0.242 to 0.396 (*restricted*) and -0.259 to 0.393 (all) with an average relatedness value among all 55 pairwise combinations of 0.073 (*std. err.* = 0.019) (*restricted*) and 0.065 (*std. err.* = 0.019) (all). If the M2583944 / M2583948 pair was related at the level of half-sibs, they would have to be related via paternal lines only as they do not share the same maternal lineage.



**Table V.18** Pairwise relatedness values for Group NS1. R-values based on the *restricted* set of allele frequencies are presented in italics.

Hap.	Individual	M2583941	M2583942	M2583943	M2583944	M2583945	M2583946	M2583947	M2583948	M2583949	M25839410	M25839411
C	M2583941	—										
C	M2583942	-0.021 <i>-0.010</i>	—									
A	M2583943	-0.007 <i>0.009</i>	0.243 <i>0.253</i>	—								
A	M2583944	-0.027 <i>-0.019</i>	-0.040 <i>-0.034</i>	0.218 <i>0.226</i>	—							
A	M2583945	-0.124 <i>-0.117</i>	-0.225 <i>-0.222</i>	-0.117 <i>-0.110</i>	0.020 <i>0.020</i>	—						
C	M2583946	0.226 <i>0.241</i>	-0.043 <i>-0.027</i>	0.081 <i>0.101</i>	-0.123 <i>-0.108</i>	-0.026 <i>-0.013</i>	—					
B	M2583947	-0.043 <i>-0.026</i>	-0.259 <i>-0.242</i>	-0.094 <i>-0.072</i>	-0.066 <i>-0.055</i>	-0.036 <i>-0.026</i>	0.055 <i>0.076</i>	—				
C	M2583948	-0.004 <i>-0.005</i>	0.163 <i>0.014</i>	-0.005 <i>-0.004</i>	0.393 <i>0.389</i>	0.082 <i>0.075</i>	0.006 <i>0.012</i>	0.134 <i>0.134</i>	—			
C	M2583949	0.026 <i>0.040</i>	0.083 <i>0.094</i>	0.070 <i>0.087</i>	0.211 <i>0.218</i>	-0.011 <i>-0.002</i>	0.112 <i>0.130</i>	0.086 <i>0.104</i>	0.310 <i>0.310</i>	—		
C	M25839410	-0.016 <i>-0.005</i>	-0.132 <i>-0.122</i>	-0.057 <i>-0.042</i>	0.075 <i>0.081</i>	0.106 <i>0.111</i>	0.064 <i>0.080</i>	0.158 <i>0.171</i>	0.276 <i>0.274</i>	0.289 <i>0.298</i>	—	
C	M25839411	0.053 <i>0.070</i>	0.140 <i>0.153</i>	0.054 <i>0.074</i>	0.225 <i>0.234</i>	0.020 <i>0.032</i>	0.167 <i>0.186</i>	0.156 <i>0.175</i>	0.393 <i>0.396</i>	0.335 <i>0.350</i>	0.039 <i>0.054</i>	—

Group NS2 contained six males (Table V.19). Five shared haplotype 'A', while one whale carried the 'B' haplotype. The group estimates of relatedness were  $-0.005$  (*restricted*) and  $-0.006$  (all). Pairwise relatedness estimates among group individuals ranged from  $-0.188$  to  $0.194$  (*restricted*) and  $-0.196$  to  $0.188$  (all). The average relatedness level was  $-0.008$  (std. err. =  $0.032$ ) and  $0.003$  (std. err. =  $0.030$ ) for the *restricted* and all datasets respectively.

Highly related pairs were not expected within prospective bachelor groups, but were tested for completeness. Two related pairs of whales were identified within the NS1 stranded group and one related pair was found within the NS2 group (Table V.20). The M0143962 / M0143966 pair that tested positive for the likelihood of full-sibling relatedness disagrees with a relation via maternal lines as they do not share the same maternally inherited mtDNA haplotype. However, the pair may share the same father and thus be related at the level of half-siblings.

### V.3.3.2 NSEA males within groups vs. GOM females within groups

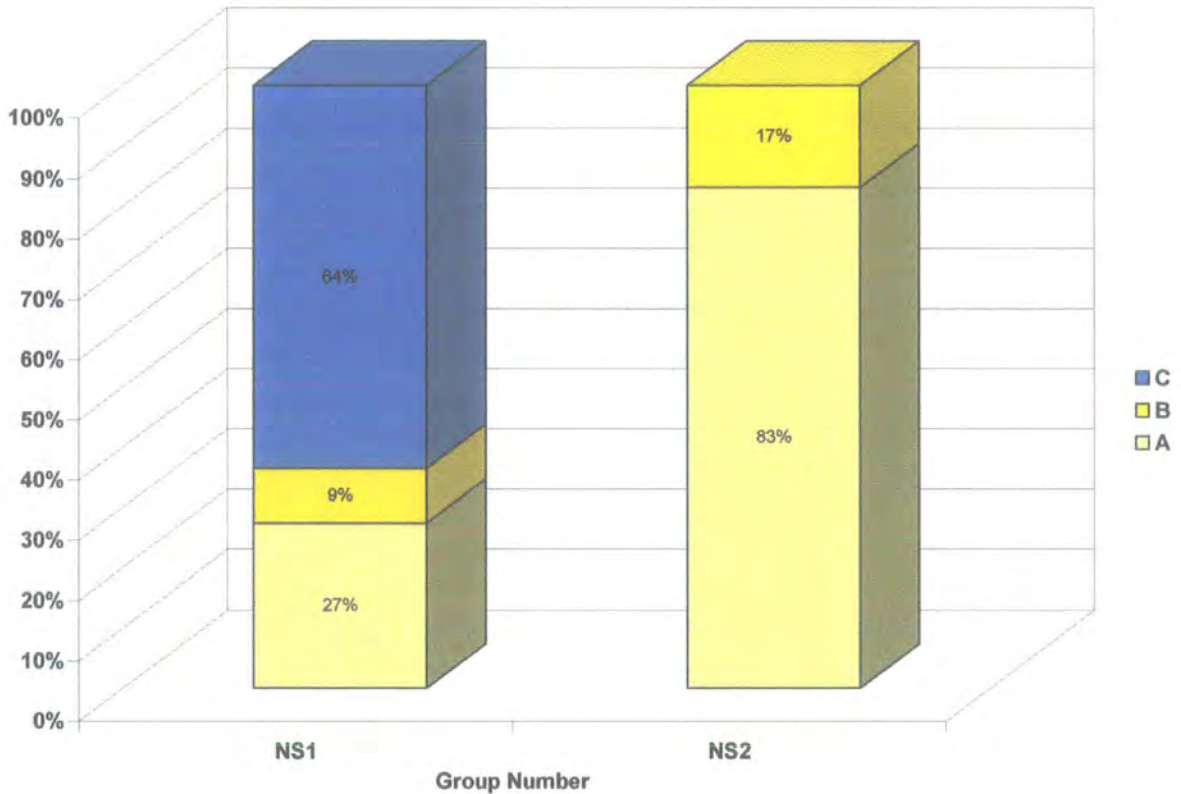
To determine whether relatedness levels among all male 'bachelor' groups are similar or different to the primarily mixed sex social groups encountered in the northern GOM, the R-values for all pairwise relatedness comparisons possible within groups NS1 and NS2 ( $N=70$ ; mean R-value =  $0.058$  and  $0.047$ ) were compared with the R-values for whales found within the groups G0-G18 ( $N=139$ ; mean R-value =  $0.063$  and  $0.054$ ) located in the northern GOM. No significant difference (*restricted* Wilcoxon-Mann-Whitney:  $U = 4666$ ,  $p = 0.631_{20,000}$ ; all Wilcoxon-Mann-Whitney:  $U = 4685$ ,  $p = 0.668_{20,000}$ ) was determined when the NSEA R-values within groups were compared to the GOM within group R-values. This suggests that males within the two groups of NSEA stranded whales are not significantly more or less related than whales found within groups G0-G18 located in the northern GOM.

**Table V.19** Pairwise relatedness values for Group NS2. R-values based on the *restricted* set of allele frequencies are presented in italics.

Hap.	Individual	M0143961	M0143962	M0143963	M0143964	M0143965	M0143966
A	M0143961	_____					
B	M0143962	-0.006 <i>-0.006</i>	_____				
A	M0143963	-0.136 <i>-0.116</i>	-0.060 <i>-0.037</i>	_____			
A	M0143964	0.118 <i>0.121</i>	-0.196 <i>-0.188</i>	-0.031 <i>-0.007</i>	_____		
A	M0143965	-0.118 <i>-0.112</i>	-0.009 <i>-0.001</i>	-0.177 <i>-0.148</i>	0.149 <i>0.159</i>	_____	
A	M0143966	0.154 <i>0.154</i>	0.052 <i>0.054</i>	-0.086 <i>-0.063</i>	0.033 <i>0.040</i>	0.188 <i>0.194</i>	_____

### V.3.3.3 Matriline composition within bachelor groups

The group of eleven whales stranded on the Orkney coast (NS1) consisted of multiple haplotypes among its members. Three whales shared the 'A' haplotype, one whale carried the 'B' haplotype and seven whales possessed the 'C' haplotype. The six whales stranded on the Grampian coast (NS2) consisted of two lineages; one whale with haplotype 'B' and five whales with haplotype 'A' (Figure V.6).



**Figure V.6** Percentage of haplotypes 'A', 'B' and 'C' occurring within two NSEA group strandings.

**Table V.20** Potential relatives within stranded groups. Italicised relationships tested (*YES/NO*) were based on the *restricted* set of allele frequencies.

First Order Relatives	Sex	Hap	Group	# Seen / # Sampled	Loci With Shared Alleles			Relationship Tested		
					2 Alleles	1 Allele	0 Alleles	Parent-Offspring	Full-Sibs	Half-Sibs Mat. / Pat.
M2583944 / M2583948	M/M	A/C	NS1	11 / 11	3	10	3	NO	NO	YES <sup>+</sup> / YES <sup>+</sup>
								<i>NO</i>	<i>NO</i>	<i>YES<sup>+</sup> / YES<sup>+</sup></i>
M2583949 / M25839410	M/M	C/C	NS1	11 / 11	2	11	3	NO	NO	YES <sup>+</sup> / YES <sup>+</sup>
								<i>NO</i>	<i>NO</i>	<i>YES<sup>+</sup> / YES<sup>+</sup></i>
M0143962 / M0143966	M/M	B/A	NS2	6 / 6	1	10	5	NO	YES <sup>+</sup>	YES <sup>++</sup> / YES <sup>+</sup>
								<i>NO</i>	<i>NO</i>	<i>YES<sup>+</sup> / YES<sup>+</sup></i>

<sup>+</sup>Significance value ( $p < 0.01$ ), <sup>++</sup>Significance value ( $p < 0.001$ )

## V.4 DISCUSSION

### V.4.1 Genetic Composition of GOM Groups

A primary goal of this study was to provide a preliminary understanding of sperm whale group composition in the northern GOM for both gender and kinship. My interpretation will be limited by the lack of long-term association data and incomplete group sampling. However, the results were comparable between poorly and well-sampled (>50%) groups.

Researchers have hypothesized that sperm whale groups in the northern GOM contain adult females, immatures and calves of both sexes (Davis et al. 1998; Weller et al. 2000; Würsig et al. 2000). My results support this suggestion. While the majority of sampled groups fit this classic mixed sex social group structure, there were three occasions when only young males were sampled from groups that contained relatively few individuals at the time of sampling. Although these may be ephemeral sub-groups from an undetected nearby larger mixed group, this unexpected result suggests the low-latitude existence of bachelor groups in the northern GOM during at least the summer months. Overall, the unequal sex ratio and observations that whales were rarely sighted alone leads us to conclude that primarily mixed sex groups were encountered in the northern GOM.

In most group-living mammals, related females form the stable interior of the social unit, while males transfer between groups (Packer 1979; Greenwood 1980). A significant degree of matrilineal group structure is believed to exist in several species of large mammals (ex. orca (Bigg et al. 1990; Baird 2000), pilot whales (Amos et al. 1991, 1993) and African elephants (Weilgart et al. 1996)) including sperm whales (Whitehead 2003). However, the fact that sperm whales possess an unusually low level of mtDNA diversity on a global scale (Lyrholm and Gyllenstein 1998) may mask the true extent of matrilineal diversity within a group. In any event, results shown here provide additional support that free-ranging groups often contain more than one matriline among group members and even though groups did contain highly related whales, not all individuals within a group were related. Previous studies involving genetic structure of sperm whale group (Richard et al. 1996a; Lyrholm et al. 1999; Bond 1999; Mesnick 2001) and unit (Christal 1998) members agree with this result and suggest that immigration and/or emigration may be occurring.

First-order kin (i.e. mother-offspring and full-siblings) were infrequent. This is again consistent with other sperm whale studies (Richard et al. 1996a; Christal 1998; Bond 1999). The most common relationship among highly related whales was that of half-siblings which is similar to what Bond (1999) described for mixed sex groups in the Azores. However, half-siblings are indistinguishable from grandparent-grandchild relationships. This result may be misleading though as relationships that were significant for both full-sibling and/or parent-offspring were also significant for half-siblings under certain circumstances. High R-values for whales with different mtDNA haplotypes suggests pairs of whales within groups related through a common paternal line. Similar putative relative pairs were found among sperm whale group members in the North Atlantic (Bond 1999) and North Pacific (Richard et al. 1996a), although mtDNA was not incorporated in Bond's (1999) study. Oestrus synchronization among female sperm whales (Best and Butterworth 1980b), similar to humpback whales (Clapham 2000) and gray whales (Jones et al. 1984), may provide important functions for both the adult females and resulting calves involved (see Whitehead and Mann 2000). Large males are thought to roam in search of groups of oestrus females (Whitehead 1990; 1993; Whitehead and Weilgart 2000), which should result in a promiscuous mating system. Taken together, paternal half-siblings seem entirely plausible. Whether the same males return to the same areas to breed is unknown. Although small calves (less than a year old) have been seen in the GOM, we have yet to see or sample a large physically and sexually mature male thought to be a successful breeder (Best 1984) in the northern Gulf during any fieldwork seasons.

The extremely low level of relatedness within female mixed sex groups was surprising. However, the difficulties involved with total group sampling may have substantially affected my group relatedness results; although poorly and well-sampled ( $\geq 50\%$ ) group results were quite comparable. In other areas, groups are known to come together and split apart on a frequent basis (Whitehead et al 1991; Richard et al. 1996a; Christal 1998). Perhaps our sampling effort reflects occasions when two or more groups (comprised of one or more units each) had congregated on a common feeding ground or during a social bout. Another hypothesis by Christal

(1998) suggests that perhaps normal patterns of genetic relatedness within units studied off the Galapagos were disrupted by whaling operations, resulting in the merger of multiple units and groups after a whaling attack.

Unfortunately, in the GOM long-term associations among groups of individuals are unknown at this stage and units believed to make up groups are not visible without long-term data. Future work must build on the combination of photoID, photogrammetry and biopsy sampling in order to combine relatedness issues with association patterns over long time periods. The incorporation of satellite-monitored tagging and biopsy sampling will provide an in-depth examination of how related and non-related whales sampled from the same group either move apart or stay together through space and time. This combination of techniques will provide an extremely fine-scale assessment into the daily lives of sperm whales.

#### **V.4.2 Genetic Composition of GOM Clusters**

Sperm whales logging side-by-side in a cluster formation at the surface are often observed during resting and socializing events as well as between foraging dives. Whitehead (2003) proposed that clusters are formed to reduce the risks of predation and to maintain bonds. Although this study merely touches on this topic and lacks the association data required for a more thorough examination, our results provide an insight into how temporary clusters may be structured from a genetic perspective. The majority of whales found clustered together were females, although cases for both male-female and all-male (a pair of immatures) clusters were found in the northern GOM. This is expected given that the majority of whales sampled in the GOM are females. The all-male cluster was sampled from what was believed to be a bachelor group in the northern GOM. Similar clustering among all-male aggregations, although less common than among mixed group members, has been witnessed off Kaikoura, over the Scotian Shelf, off Andenes and around the Galapagos Islands (Whitehead et al. 1992a; Childerhouse et al. 1995; Christal and Whitehead 1997; Lettevall et al. 2002). The majority of GOM clusters contained members that shared a single haplotype, although cases where clusters contained up to three haplotypes among members were found. Relatedness among clustered whales was not significantly higher than for whales found in groups.



### V.4.3 Genetic Composition of North Sea Strandings

Bond's (1999) examination of relatedness for two bachelor groups revealed that both groups were mainly comprised of unrelated whales, although within each stranding at least one half-sibling pair was found among members. Our results incorporated mtDNA sequencing as well as additional polymorphic microsatellites for the same two groups and confirmed Bond's (1999) findings. Relatedness levels among members were not significantly different than those calculated from whales within groups in the northern GOM that primarily contained females and young of both sexes. Both strandings were composed of two or more lineages so relatedness via maternal lines was not always plausible for pairwise comparisons. This was true with respect to one of the two half-sibling pairs in the NS1 group as well as the one half-sibling pair in the NS2 group. The three half-sibling pairs found within the two groups were most likely related through common paternal lines. An important note is that our two aggregations of stranded individuals, in addition to a similar stranding event in Nova Scotia where a male sperm whale intentionally stranded within 50 m of two other recently stranded whales (Lucas and Hooker 2000), have no long-term data to base associations patterns between individuals on and may not portray an accurate representation of what occurs among free-ranging all-male aggregations.

All-male groups formed after dispersal from their natal groups have been documented in terrestrial mammals (e.g. lions: *Panthera leo* (Bygott, et al. 1979) and Japanese macaques: *Macaca fuscata* (Sugiyama 1976)). The formation of aggregations, or bachelor groups, by young male sperm whales after dispersal from their natal groups is strikingly similar to what's been considered the sperm whale's terrestrial mammalian counterpart, the African elephant (*Loxodonta africana*) (Best 1979; Weilgart et al. 1996; Douglas-Hamilton et al. 2001). Both species fit the typical mammalian pattern of dispersion where females are philopatric and males disperse (Greenwood 1980). Previous studies show that sexually mature or maturing male sperm whales showed no evidence for preferred companionship and long-term relationships amongst individuals (Lettevall et al. 2002), which is stark contrast to that observed by female-based mixed groups (Whitehead et al. 1991). Also interesting is that males within aggregations have been shown to coordinate their headings and even cluster together, although far less common than that observed for female groups (Childerhouse et al. 1995; Lettevall et al. 2002). This suggests that

males do respond to each other, even though they appear to lack long-term associates (Lettevall et al. 2002). As previously mentioned, tight knit female-based mixed groups may arise in response to communal care of calves, defence against predators and cooperative foraging strategies (Whitehead and Weilgart 2000). However, young male aggregations don't contain calves, so care of calves is not required among members. Although tooth rake marks are visible on all ages of males and females, documented attacks by orca and harassment by pilot whales have only been observed for female-based mixed groups (Arnbom et al. 1987; Weller et al. 1996; Pitman and Chivers 1999). Finally, cooperative foraging among young males within an area seems unlikely given the more dispersed nature of these aggregations (Lettevall et al. 2002).

## ***VI. Summary of Results and Recommendations for Future Research***

The research described in this thesis provides a detailed understanding of the molecular ecology for sperm whales occupying the northern Gulf of Mexico (GOM) in addition to describing genetic details for the putative populations located in the Mediterranean Sea (MED), North Sea (NSEA) and North Atlantic Ocean (NAO). Population structuring between geographic locations, with respect to mtDNA, was highly significant and warrants the classification of each putative population as unique stocks for management purposes. The genetic composition of GOM sperm whale groups fits the previously described scenarios for both mixed sex and bachelor groups located in other areas of the world, while the two groups from the NSEA stranding fit the bachelor group scenario. Surface behavioural reactions to biopsy darting were primarily mild and short-term.

### **VI.1 GENETIC STRUCTURE OF THREE PUTATIVE GEOGRAPHIC SPERM WHALE POPULATIONS**

The northern GOM stock is currently listed as a separate stock from that of the western NAO (Waring et al. 2001). At present, this appears to be based solely on geographic boundaries. Prior to this research, distribution and abundance surveys found sperm whales present year-round throughout the northern GOM which may be an indication of philopatry by some whales to an area (Davis et al. 1998). Molecular sexing results indicate that the majority of samples obtained from the Gulf were from immature and adult female whales. If the expected pattern for mammalian dispersion (Greenwood 1980) holds true and females are philopatric to particular geographic areas as indicated by surveys and resightings of individuals within an area over time (Weller et al. 2000), then population structuring with respect to the maternally inherited mtDNA genome may be visible between putative populations (barring extensive emigration and immigration between geographic locations). Although variation and diversity between locations were low, the highly significant level of structuring with respect to this examination of 399 bp of the mtDNA control region supports previous genetic results suggesting a significant degree of female philopatry between ocean basins (Lyrholm and Gyllenstein 1998). This study's

comparison of mtDNA haplotypes between regions proved highly significant with respect to genetic differentiation measures ( $F_{ST}$  range = 0.279 (*restricted*) to 0.539 (all);  $\Phi_{ST}$  range = 0.333 (*restricted*) to 0.485 (all)) and was consistent with Lyrholm and Gyllenstein (1998) results which provided evidence of mitochondrial genetic differentiation on a world-wide scale. This is not specific to sperm whales though and has been described for a variety of other marine mammals (see Chapter 4 and Hoelzel et al. 2002*b* for a comparative review). While Lyrholm's global-scale study found low mtDNA variation between oceans, this study provides a novel finding in the form of two unique haplotypes ('X' and 'Y') only found among whales sampled in the northern GOM. The majority of whales sampled in the GOM carry Haplotype 'X'. Also unique was the fact that all samples sequenced from the MED contained only one haplotype ('C'). Although haplotype 'C' was the most common haplotype among all the NAO samples, the total lack of haplotype and nucleotide diversity within the MED sample set may be an indication of population isolation, small effective population size or bottleneck event that has reduced maternal lineages (Baker et al. 1999; Hoelzel et al. 2002*a*; Lyrholm et al. 1996).

Also in agreement with Greenwood (1980), the lack of strong significant nuclear differentiation between neighbouring populations suggests that sexually mature males disperse from their natal populations and spread their genes to the more philopatric females.  $F_{ST}$ ,  $R_{ST}$  and  $Rho_{ST}$  values ( $< 0.08$ ), although significant, indicated minimal genetic differentiation between the GOM-MED and the MED-NSEA (significant for  $F_{ST}$  only) populations with respect to nuclear differentiation. By testing for sex-biased dispersal, our  $F_{IS}$ ,  $H_S$  and mean assignment results were all in agreement with males being the dispersers and females being the more site-faithful of the sexes. However, larger sample sizes are required to increase the power of these tests (Goudet et al. 2002). While Lyrholm et al. (1999) has already provided evidence for sex-biased dispersal occurring on a global scale, our sample set compares a more restricted geographic area and only includes populations that border the NAO.

Overall, our population structure results support the delineation of the northern GOM into a female-dominated stock that is genetically distinct from the NAO, MED and NSEA putative populations. As such, the GOM population requires proper management to ensure stock survival. While the putative population in the

MED lacks any mtDNA variation among sampled members, only further sampling in conjunction with additional studies focusing on contaminant analysis, site-fidelity, movement patterns and habitat use will provide a more thorough understanding with regards to questions of isolation.

### **VI.1.1 Recommendations for Future Research – Population Structure**

A continuation and extension of multi-faceted research techniques in multiple locations (e.g. the southern Gulf of Mexico, the Caribbean Sea, the MED, throughout the western and eastern NAO and NSEA) would provide further support needed to accurately describe levels of both population and possibly subpopulation structuring. Movement among female sperm whales appears to be limited resulting in population structuring with respect to the mtDNA genome. The ability to ‘bridge the gaps’ between geographic areas would provide valuable information as to the extent of gene flow within and among geographic locations as well as provide manageable stock boundaries. The development and incorporation of Y-chromosome genetic markers would allow for an assessment of whether genetic variation between males from different geographic populations exists in addition to quantifying levels of relatedness between males from multiple geographic locations. Dedicated efforts to sample large physically and sexually mature males generally found at higher latitudes as well as at lower latitudes during the breeding season would provide further clues as to how males disperse from their natal populations and spread their genes to the more philopatric females. The compilation of genetic studies via collaboration amongst researchers around the globe provides important answers with regards to previously unknown questions. Calibrations are currently underway to combine our microsatellite allele size results with those of published data (i.e. Lyrholm et al. 1999) on sperm whale microsatellites to provide a more detailed picture as to how these three putative populations fit into the global sperm whale nuclear DNA picture. Further sampling in conjunction with additional studies focusing on contaminant analysis, site-fidelity, movement patterns, habitat use and coda structure among clans will provide a proper understanding of how to properly manage existing sperm whale populations.

## **VI.2 COMPOSITION OF SPERM WHALE GROUPS AND CLUSTERS IN THE NORTHERN GULF OF MEXICO AND THE NORTH SEA**

Based on preliminary length estimates and group size estimates conducted during the GulfCet I and GulfCet II cruises, sperm whale groups encountered in the northern GOM were assumed to contain adult females, immatures and calves of both sexes (Davis et al. 1998; Weller et al. 2000; Würsig et al. 2000). In order to accurately assess group type and relatedness among whales within and between groups, this study compared a greater number of polymorphic microsatellites ( $N = 16$ ) than previous sperm whale studies, analyzed the highly variable mtDNA control region to describe maternal lineages and incorporated gender results based on molecular sexing techniques. Although our assessment of group composition lacks the required long-term association data and total group sampling to fully understand social structure within GOM groups, both poorly and well-sampled ( $\geq 50\%$ ) group results were quite comparable with gender and relatedness findings suggesting that the majority of groups encountered in the GOM fit the mixed-sex group scenario comprised of both related and unrelated adult females and young of both sexes. The occurrence of what seems to be all-male bachelor groups utilizing the same low-latitude feeding grounds as the female mixed-sex groups in the GOM was unexpected.

Relatedness within groups was surprisingly low, but significantly greater than relatedness found between groups. This result is consistent with other sperm whale studies that have focused on both groups and units (Richard et al. 1996a; Christal 1998; Bond 1999). There were instances of first-order kin pairs present among sampled group members; however, they were not as frequent as one would expect within a previously described matrilineal species where females show high levels of care for their offspring (Whitehead and Weilgart 2000). Interestingly, groups were composed of both single and multiple (up to 3 in some instances) matrilineal lines. Individuals that shared numerous alleles across multiple loci, but carried different mtDNA haplotypes were assumed to be related at the level of half-siblings via a common paternal line or perhaps grandmother/grandchild (no large males that could be grandfathers were present in the study site) if they shared the same haplotype. Bond (1999) described half-sibling relationships as the most common for mixed sex groups in the Azores and our findings for the GOM appear quite similar. However,

the Azores groups were predominantly composed of related individuals (Bond 1999) while the GOM group relatedness values imply that groups are primarily composed of unrelated members.

The sampling of clusters was undertaken on an opportunistic basis. Clusters contained both single and multiple (up to 3) haplotypes and relatedness results among clustered members indicated that clustered whales were no different than whales found within groups.

Our comparison of two bachelor groups of roughly the same age stranded in the NSEA confirmed Bond's (1999) results indicating group members were primarily unrelated, although potential half-sibling pairs were present within each group. Both groups were composed of more than one lineage. Surprisingly, group relatedness for these two bachelor groups was not statistically different than for groups comprised of mixed sex and all-male members located in the GOM. Caution should always be taken when interpreting results from standings due to the nature of event.

### **VI.2.1 Recommendations for Future Research – Group Composition**

Unfortunately, the GOM lacks long-term association data required to accurately describe social affiliations among group members. Our data is unable to imply whether whales sampled within groups are constant companions or simply casual acquaintances that mix with permanent group members on a temporary basis (Whitehead et al. 1991). Future work must build on the integration of photoID, photogrammetry and biopsy sampling in order to combine relatedness issues with association patterns between whales of an accurately known age class over long durations. The incorporation of satellite-monitored tagging and biopsy sampling will provide one of the most in-depth examinations of how related and non-related whales sampled within a group either move apart or stay together through space and time. This combination of techniques promises an extremely fine-scale assessment into the daily lives of sperm whales utilizing the northern GOM.

### **VI.3 SURFACE REACTIONS TO BIOPSY SAMPLING**

An assessment of behavioural reactions to biopsy sampling showed that the majority of reactions were generally mild and short-term “startle” responses similar to those described by Whitehead et al. (1990). No significant difference in behavioural reactions was observed between males and females. Although my sample size was small, no visible reactions to biopsy darting of the flukes were noticed and repeat biopsy events on the same individuals did not lead to increasing responses. Overall, these results are in agreement with previous studies performed on numerous cetacean species including sperm whales.

#### **VI.3.1 Recommendations for Future Research – Biopsy Sampling**

Although alternate techniques to gather DNA from free-ranging whales are possible (e.g. the collection of free-floating sloughed skin), the quality and quantity of DNA recovered can be difficult to work with and unreliable in some instances. However, sloughed skin has been shown to provide sufficient DNA for both mtDNA and nuclear DNA analyses and should be collected when the opportunity arises in areas where biopsy darting is not permitted. The combination of photoID, photogrammetry and fluke biopsy sampling allows the researcher to gather as much information as possible from free-ranging whales. Recommendations are to support sperm whale fluke biopsy sampling in conjunction with photoID and photogrammetry by experienced researchers using the previously described techniques.



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## *Appendix I*

### *Distances Between Re-Sampling Events*

Table A.1 GOM number, sample # (provides the date and number of sample for that day (e.g. 00070201 was taken in the year 2000 (00), in the month of July (07), on the second day in July (02) and is the first sample collected that day (01). Superscripts are provided for samples that have multiple duplicates (>2) for comparison purposes.

GOM #	Sample #	Latitude (°N)	Longitude (°W)	Distance (km)
GOM001	00070201	27.854	89.613	
GOM002	00071301	28.143	89.530	
GOM002	00071302	28.142	89.530	0.11
GOM003 <sup>a</sup>	00070501	27.708	90.179	
GOM003 <sup>b</sup>	00070502	27.708	90.179	0.00 <sup>a</sup>
GOM003 <sup>c</sup>	00070503	27.708	90.179	0.00 <sup>a</sup> , 0.00 <sup>b</sup>
GOM003 <sup>d</sup>	00071601	28.088	89.727	0.00 <sup>a</sup> , 0.00 <sup>b</sup> , 61.26 <sup>c</sup>
GOM004	00071602	28.088	89.727	
GOM005	00071603	28.041	89.768	
GOM006	00071604	28.041	89.774	
GOM007	00071605	28.036	89.778	
GOM008	00071701	28.046	89.727	
GOM008	00071705	27.983	89.774	8.38
GOM009	00071702	28.046	89.727	
GOM009	00071704	27.981	89.770	8.36
GOM010	00071703	27.990	89.738	
GOM011	00071901	28.495	89.061	
GOM012 <sup>e</sup>	00071904 (1)	28.553	88.992	
GOM012 <sup>f</sup>	00071904 (2)	28.553	88.992	0.00 <sup>e</sup>
GOM012 <sup>g</sup>	00071904 (3)	28.553	88.992	0.00 <sup>e</sup> , 0.00 <sup>f</sup>
GOM012 <sup>h</sup>	02070307	28.805	88.698	0.00 <sup>e</sup> , 0.00 <sup>f</sup> , 40.07 <sup>g</sup>
GOM013	00071905	28.556	88.989	
GOM014 <sup>i</sup>	00071902 (1)	28.553	88.985	
GOM014 <sup>j</sup>	00071903 (1)	28.555	88.985	0.22 <sup>i</sup>
GOM014 <sup>k</sup>	00071906	28.553	88.990	0.49 <sup>i</sup> , 0.54 <sup>j</sup>
GOM014 <sup>l</sup>	02070701	28.701	88.665	35.28 <sup>i</sup> , 35.18 <sup>j</sup> , 35.71 <sup>k</sup>
GOM015	00071907	28.552	88.992	
GOM015	02070302	28.705	88.764	27.99
GOM016	00071908	28.554	88.993	
GOM016	02070201	28.866	88.496	59.57
GOM017	00071909	28.554	88.998	
GOM017	02070301	28.794	88.808	32.47

Table A.1 (cont.)

GOM #	Sample #	Latitude (°N)	Longitude (°W)	Distance (km)
GOM018 <sup>m</sup>	00072001	28.663	88.659	
GOM018 <sup>n</sup>	00072003	28.722	88.723	9.05 <sup>m</sup>
GOM018 <sup>o</sup>	00072004	28.724	88.726	9.41 <sup>m</sup> , 0.37 <sup>n</sup>
GOM019	00072002	28.693	88.719	
GOM020	00072005	28.731	88.729	
GOM021	00072006	28.762	88.726	
GOM022 <sup>p</sup>	00072101	28.500	88.815	
GOM022 <sup>q</sup>	00072103	28.583	88.801	9.32 <sup>p</sup> ,
GOM022 <sup>r</sup>	00072104	28.592	88.801	10.31 <sup>p</sup> , 1.00 <sup>q</sup>
GOM023	00072102	28.583	88.803	
GOM023	02082401	28.221	89.405	71.28
GOM024	00072105	28.592	88.798	
GOM025	00070701	28.693	88.835	
GOM025	00072106	28.592	88.798	11.79
GOM026	00070702	28.684	88.861	
GOM026	00072107	28.613	88.794	10.24
GOM027	00072401	28.841	87.168	
GOM028	00072402	28.822	87.141	
GOM029	00072603	29.169	87.362	
GOM030	00072604	29.185	87.398	
GOM031	00051406	24.480	83.966	
GOM032	00052802	27.500	91.400	
GOM033	01031701	24.684	84.127	
GOM034	01031702	24.683	84.185	
GOM035	01031703	24.691	84.222	
GOM036	01031704	24.701	84.232	
GOM037	01031705	24.689	84.257	
GOM038	01032601	25.277	84.928	
GOM039 <sup>s</sup>	01032602	25.286	84.891	
GOM039 <sup>t</sup>	01032605	25.251	84.898	3.95 <sup>s</sup>
GOM039 <sup>u</sup>	01032606	25.259	84.902	3.20 <sup>s</sup> , 0.98 <sup>t</sup>
GOM040	01032603	25.253	84.903	
GOM040	01032604	25.249	84.909	0.75
GOM041	01032607	25.260	84.911	
GOM042	01032801	24.625	84.201	
GOM043	01040101	28.073	85.711	
GOM044	01040102	28.099	85.721	

Table A.1 (cont.)

GOM #	Sample #	Latitude (°N)	Longitude (°W)	Distance (km)
GOM045 <sup>y</sup>	00070703	28.683	88.860	
GOM045 <sup>w</sup>	01071901	28.817	88.694	21.98 <sup>y</sup>
GOM045 <sup>x</sup>	02082302	28.301	89.519	77.09 <sup>y</sup> , 98.84 <sup>w</sup>
GOM045 <sup>y</sup>	02082303	28.330	89.664	87.76 <sup>y</sup> , 109.03 <sup>w</sup> , 14.55 <sup>x</sup>
GOM046	01072301	28.883	88.576	
GOM047	01072601	29.319	87.380	
GOM047	01072702	29.280	87.320	7.25
GOM048 <sup>y</sup>	00072601	29.186	87.440	
GOM048 <sup>z</sup>	00072602	29.158	87.422	3.57 <sup>y</sup>
GOM048 <sup>aa</sup>	01072701	29.272	87.305	16.21 <sup>y</sup> , 17.00 <sup>z</sup>
GOM049	01072801	28.895	88.269	
GOM049	01080101	29.064	88.266	18.78
GOM050	01072802	28.895	88.269	
GOM050	01072805	29.020	88.174	16.68
GOM051	01072803	28.824	88.301	
GOM051	01072804	28.856	88.292	3.66
GOM052	01080804	29.162	87.738	
GOM053 <sup>bb</sup>	01080701	29.041	88.054	
GOM053 <sup>cc</sup>	01080805	29.157	87.802	27.66 <sup>bb</sup>
GOM053 <sup>dd</sup>	01080806	29.178	87.800	28.98 <sup>bb</sup> , 2.34 <sup>cc</sup>
GOM053 <sup>ce</sup>	02062801	29.208	87.178	87.03 <sup>bb</sup> , 60.80 <sup>cc</sup> , 60.43 <sup>dd</sup>
GOM054	01081301	28.902	88.375	
GOM055 <sup>ff</sup>	01081302	28.939	88.331	
GOM055 <sup>gg</sup>	01081303	28.947	88.330	0.89 <sup>ff</sup>
GOM055 <sup>hh</sup>	01081304	28.947	88.334	0.94 <sup>ff</sup> , 0.39 <sup>gg</sup>
GOM055 <sup>ii</sup>	02082501	28.780	88.693	39.40 <sup>ff</sup> , 39.90 <sup>gg</sup> , 39.56 <sup>hh</sup>
GOM055 <sup>jj</sup>	02082801	28.651	89.005	73.02 <sup>ff</sup> , 73.50 <sup>gg</sup> , 73.15 <sup>hh</sup> , 33.62 <sup>ii</sup>
GOM056	01081305	29.004	88.347	
GOM056	01081306	28.999	88.349	0.59
GOM057	01081401	28.790	88.804	
GOM058 <sup>kk</sup>	01081402	28.795	88.799	
GOM058 <sup>ll</sup>	01081403	28.795	88.789	0.97 <sup>kk</sup> ,
GOM058 <sup>mm</sup>	01081409	28.771	88.704	9.63 <sup>kk</sup> , 8.70 <sup>ll</sup>
GOM058 <sup>nn</sup>	02082601	28.686	88.944	18.61 <sup>kk</sup> , 19.36 <sup>ll</sup> , 25.22 <sup>mm</sup>

Table A.1 (cont.)

GOM #	Sample #	Latitude (°N)	Longitude (°W)	Distance (km)
GOM059 <sup>oo</sup>	01081404	28.801	88.699	
GOM059 <sup>pp</sup>	01081405A	28.801	88.699	0.00 <sup>oo</sup>
GOM059 <sup>qq</sup>	01081405B	28.801	88.699	0.00 <sup>oo</sup> , 0.00 <sup>pp</sup>
GOM059 <sup>rr</sup>	02082301	28.239	89.562	104.87 <sup>oo</sup> , 104.87 <sup>pp</sup> , 104.87 <sup>qq</sup>
GOM060	01081406	28.802	88.688	
GOM061	01081407	28.788	88.693	
GOM062	01081408	28.771	88.704	
GOM063 <sup>ss</sup>	01081504	28.165	89.343	
GOM063 <sup>tt</sup>	01081505	28.160	89.344	0.56 <sup>ss</sup> ,
GOM063 <sup>uu</sup>	01081804	28.114	89.548	20.87 <sup>ss</sup> , 20.63 <sup>tt</sup>
GOM064	01081506	28.155	89.346	
GOM065	01081507	28.153	89.345	
GOM065	01081803	28.146	89.149	19.22
GOM066	01081508	28.147	89.342	
GOM067	01081601	28.038	89.881	
GOM068	01081602	28.078	89.852	
GOM069	01081603	28.096	89.780	
GOM070	01081604	28.105	89.705	
GOM071	01081701	27.772	90.066	
GOM072	01081702	27.748	90.088	
GOM073	01081703	27.733	90.110	
GOM074	01081801	28.159	89.466	
GOM075	01081802	28.143	89.474	
GOM076	01081805	28.103	89.547	
GOM076	02070601	28.426	89.050	60.45
GOM077	01081806	28.099	89.620	
GOM078	01081807	27.960	89.644	
GOM079	01081808	27.910	89.705	
GOM080	02062401	28.056	89.669	
GOM081	02062402	28.012	89.696	
GOM082	02070101	28.958	88.109	
GOM083	02070102	28.956	88.109	
GOM084	02070103	28.951	88.113	
GOM085	02070104	28.903	88.099	

Table A.1 (cont.)

GOM #	Sample #	Latitude (°N)	Longitude (°W)	Distance (km)
GOM086	02070105	28.903	88.092	
GOM086	02070106	28.903	88.092	0.00
GOM087	02070202	28.884	88.478	
GOM087	02070308	28.808	88.684	21.76
GOM088	02070303	28.840	88.692	
GOM088	02070306	28.799	88.699	4.61
GOM089	02070304	28.850	88.673	
GOM089	02070305	28.850	88.673	0.00
GOM090	02070602	28.445	88.991	
GOM091	02070702	28.742	88.875	
GOM092	02070801	28.992	88.239	
GOM093	02082304	28.301	89.519	
GOM094	02082502	28.791	88.710	
GOM095	02082901	28.832	88.600	
GOM096	02091101	28.664	88.983	
GOM097	02091102	28.662	88.992	
GOM098	02091103	28.661	88.995	
GOM099	02091401	27.650	92.757	

## *Appendix II*

### *Allele Frequencies for Each Locus and Location*

**Table A.II.1** Allele frequencies for 16 microsatellite loci for the GOM, MED and NSEA 'all' and 'restricted' populations. Allele sizes in base pairs (bp) are shown in bold type. Private alleles have been underlined.

<b>EV1</b>	<u>Gulf of Mexico</u>		<u>Mediterranean Sea</u>		<u>North Sea</u>	
<b>Allele Size (bp)</b>	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>
<b>123</b>	0.639	0.550	0.864	0.813	0.600	0.583
<b>125</b>	0.024	0.038	0.000	0.000	0.025	0.028
<b>131</b>	0.024	0.025	0.000	0.000	0.025	0.028
<b>133</b>	0.108	0.125	0.045	0.000	0.025	0.028
<b>135</b>	0.030	0.050	0.000	0.000	0.025	0.028
<b>137</b>	0.036	0.063	0.023	0.000	0.050	0.056
<b>139</b>	0.127	0.125	0.068	0.188	0.225	0.222
<b>141</b>	0.012	0.025	0.000	0.000	0.025	0.028
<b>EV5</b>	<u>Gulf of Mexico</u>		<u>Mediterranean Sea</u>		<u>North Sea</u>	
<b>Allele Size (bp)</b>	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>
<b>155</b>	0.452	0.350	0.273	0.188	0.400	0.417
<b>157</b>	0.036	0.038	0.205	0.250	0.025	0.028
<b>159</b>	0.289	0.288	0.295	0.250	0.275	0.250
<b>161</b>	0.072	0.075	0.091	0.125	0.150	0.139
<b>165</b>	0.018	0.025	0.000	0.000	0.025	0.028
<b>167</b>	0.054	0.088	0.114	0.125	0.100	0.111
<b>169</b>	0.024	0.050	0.023	0.063	0.025	0.028
<u><b>171</b></u>	0.018	0.025	0.000	0.000	0.000	0.000
<u><b>175</b></u>	0.036	0.063	0.000	0.000	0.000	0.000
<b>EV37</b>	<u>Gulf of Mexico</u>		<u>Mediterranean Sea</u>		<u>North Sea</u>	
<b>Allele Size (bp)</b>	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>
<u><b>194</b></u>	0.006	0.013	0.000	0.000	0.000	0.000
<u><b>196</b></u>	0.006	0.013	0.000	0.000	0.000	0.000
<b>204</b>	0.030	0.050	0.048	0.000	0.000	0.000
<b>206</b>	0.181	0.150	0.048	0.063	0.250	0.222
<b>208</b>	0.102	0.100	0.071	0.063	0.075	0.083
<b>210</b>	0.090	0.113	0.000	0.000	0.125	0.139
<b>212</b>	0.006	0.013	0.095	0.000	0.050	0.056
<b>214</b>	0.042	0.025	0.238	0.313	0.125	0.111
<b>216</b>	0.078	0.063	0.024	0.000	0.075	0.083
<b>218</b>	0.102	0.100	0.143	0.188	0.025	0.028
<b>220</b>	0.054	0.025	0.000	0.000	0.075	0.083
<b>224</b>	0.102	0.150	0.048	0.000	0.050	0.056
<u><b>228</b></u>	0.006	0.000	0.000	0.000	0.000	0.000
<b>229</b>	0.012	0.013	0.000	0.000	0.025	0.028
<u><b>230</b></u>	0.000	0.000	0.000	0.000	0.025	0.028

Table A.II.1

<b>EV37 (cont.)</b>						
<b>Allele Size (bp)</b>	<u>Gulf of Mexico</u>		<u>Mediterranean Sea</u>		<u>North Sea</u>	
	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>
<u>231</u>	0.006	0.000	0.000	0.000	0.000	0.000
<u>233</u>	0.012	0.025	0.000	0.000	0.000	0.000
<u>235</u>	0.024	0.038	0.000	0.000	0.000	0.000
<u>237</u>	0.006	0.000	0.119	0.063	0.025	0.000
<u>238</u>	0.096	0.075	0.143	0.250	0.025	0.028
<u>240</u>	0.012	0.013	0.024	0.063	0.025	0.028
<u>241</u>	0.006	0.000	0.000	0.000	0.000	0.000
<u>243</u>	0.018	0.025	0.000	0.000	0.000	0.000
<u>244</u>	0.000	0.000	0.000	0.000	0.025	0.028

<b>EV94</b>						
<b>Allele Size (bp)</b>	<u>Gulf of Mexico</u>		<u>Mediterranean Sea</u>		<u>North Sea</u>	
	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>
<u>196</u>	0.000	0.000	0.000	0.000	0.025	0.028
<u>200</u>	0.018	0.038	0.000	0.000	0.025	0.028
<u>202</u>	0.283	0.250	0.136	0.188	0.200	0.194
<u>204</u>	0.271	0.225	0.205	0.188	0.300	0.250
<u>206</u>	0.181	0.150	0.068	0.063	0.150	0.167
<u>208</u>	0.048	0.075	0.250	0.188	0.100	0.111
<u>210</u>	0.042	0.050	0.000	0.000	0.050	0.056
<u>212</u>	0.036	0.063	0.136	0.125	0.100	0.111
<u>214</u>	0.042	0.050	0.023	0.000	0.025	0.028
<u>216</u>	0.012	0.025	0.023	0.000	0.025	0.028
<u>218</u>	0.006	0.013	0.159	0.250	0.000	0.000
<u>220</u>	0.036	0.038	0.000	0.000	0.000	0.000
<u>222</u>	0.006	0.013	0.000	0.000	0.000	0.000
<u>226</u>	0.018	0.013	0.000	0.000	0.000	0.000

<b>EV104</b>						
<b>Allele Size (bp)</b>	<u>Gulf of Mexico</u>		<u>Mediterranean Sea</u>		<u>North Sea</u>	
	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>
<u>158</u>	0.241	0.200	0.476	0.438	0.275	0.250
<u>160</u>	0.000	0.000	0.000	0.000	0.025	0.028
<u>162</u>	0.169	0.200	0.048	0.125	0.025	0.028
<u>164</u>	0.398	0.425	0.286	0.375	0.425	0.472
<u>166</u>	0.193	0.175	0.190	0.063	0.250	0.222

<b>SW10</b>						
<b>Allele Size (bp)</b>	<u>Gulf of Mexico</u>		<u>Mediterranean Sea</u>		<u>North Sea</u>	
	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>
<u>139</u>	0.006	0.013	0.023	0.000	0.000	0.000
<u>143</u>	0.042	0.038	0.182	0.313	0.075	0.083
<u>145</u>	0.072	0.063	0.000	0.000	0.050	0.056
<u>147</u>	0.223	0.200	0.250	0.188	0.175	0.139
<u>149</u>	0.072	0.050	0.136	0.063	0.050	0.056

Table A.II.1

SW10 (cont.)						
Allele Size (bp)	Gulf of Mexico		Mediterranean Sea		North Sea	
	All	Restricted	All	Restricted	All	Restricted
151	0.151	0.163	0.091	0.063	0.150	0.139
153	0.114	0.100	0.068	0.125	0.125	0.111
155	0.163	0.188	0.114	0.063	0.225	0.250
157	0.054	0.088	0.091	0.125	0.050	0.056
159	0.054	0.050	0.000	0.000	0.050	0.056
161	0.048	0.050	0.045	0.063	0.050	0.056

SW13						
Allele Size (bp)	Gulf of Mexico		Mediterranean Sea		North Sea	
	All	Restricted	All	Restricted	All	Restricted
134	0.018	0.025	0.000	0.000	0.025	0.028
150	0.000	0.000	0.000	0.000	0.025	0.028
156	0.006	0.013	0.000	0.000	0.000	0.000
158	0.060	0.050	0.023	0.000	0.150	0.139
160	0.072	0.088	0.136	0.125	0.075	0.083
162	0.361	0.350	0.614	0.688	0.350	0.333
164	0.169	0.188	0.182	0.125	0.125	0.139
166	0.078	0.088	0.000	0.000	0.075	0.083
168	0.145	0.138	0.045	0.063	0.175	0.167
170	0.078	0.050	0.000	0.000	0.000	0.000
174	0.012	0.013	0.000	0.000	0.000	0.000

SW19						
Allele Size (bp)	Gulf of Mexico		Mediterranean Sea		North Sea	
	All	Restricted	All	Restricted	All	Restricted
91	0.042	0.063	0.000	0.000	0.000	0.000
97	0.084	0.100	0.000	0.000	0.100	0.083
114	0.006	0.013	0.000	0.000	0.000	0.000
116	0.030	0.038	0.000	0.000	0.025	0.028
120	0.024	0.025	0.000	0.000	0.000	0.000
122	0.072	0.025	0.250	0.313	0.000	0.000
124	0.060	0.050	0.000	0.000	0.000	0.000
126	0.018	0.025	0.045	0.000	0.175	0.167
128	0.133	0.163	0.136	0.063	0.100	0.111
130	0.102	0.088	0.023	0.000	0.075	0.083
132	0.193	0.138	0.023	0.000	0.050	0.056
134	0.084	0.100	0.023	0.000	0.150	0.167
136	0.060	0.050	0.159	0.188	0.100	0.083
138	0.036	0.050	0.182	0.188	0.050	0.028
140	0.042	0.063	0.000	0.000	0.100	0.111
145	0.006	0.000	0.159	0.250	0.025	0.028
149	0.006	0.013	0.000	0.000	0.000	0.000
157	0.000	0.000	0.000	0.000	0.025	0.028
167	0.000	0.000	0.000	0.000	0.025	0.028



Table A.II.1

<b>TEXVET5</b>						
<b>Allele Size (bp)</b>	<u>Gulf of Mexico</u>		<u>Mediterranean Sea</u>		<u>North Sea</u>	
	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>
<b>196</b>	0.018	0.025	0.000	0.000	0.075	0.083
<b>198</b>	0.151	0.150	0.159	0.125	0.175	0.194
<b>200</b>	0.030	0.025	0.045	0.063	0.000	0.000
<b>202</b>	0.000	0.000	0.000	0.000	0.025	0.000
<b>204</b>	0.042	0.050	0.045	0.000	0.050	0.056
<b>206</b>	0.151	0.200	0.250	0.313	0.200	0.194
<b>208</b>	0.259	0.213	0.182	0.125	0.250	0.222
<b>210</b>	0.229	0.225	0.227	0.250	0.175	0.194
<b>212</b>	0.036	0.038	0.000	0.000	0.025	0.028
<b>214</b>	0.036	0.038	0.091	0.125	0.025	0.028
<b>216</b>	0.012	0.013	0.000	0.000	0.000	0.000
<b>218</b>	0.036	0.025	0.000	0.000	0.000	0.000

<b>D08</b>						
<b>Allele Size (bp)</b>	<u>Gulf of Mexico</u>		<u>Mediterranean Sea</u>		<u>North Sea</u>	
	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>
<b>83</b>	0.548	0.613	0.810	1.000	0.632	0.647
<b>91</b>	0.024	0.025	0.000	0.000	0.026	0.029
<b>93</b>	0.217	0.213	0.119	0.000	0.211	0.206
<b>101</b>	0.175	0.113	0.048	0.000	0.105	0.088
<b>103</b>	0.036	0.038	0.000	0.000	0.026	0.029
<b>105</b>	0.000	0.000	0.024	0.000	0.000	0.000

<b>D22</b>						
<b>Allele Size (bp)</b>	<u>Gulf of Mexico</u>		<u>Mediterranean Sea</u>		<u>North Sea</u>	
	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>
<b>107</b>	0.084	0.113	0.025	0.000	0.050	0.056
<b>109</b>	0.614	0.675	0.500	0.563	0.675	0.694
<b>111</b>	0.102	0.075	0.275	0.250	0.075	0.083
<b>113</b>	0.187	0.138	0.200	0.188	0.175	0.167
<b>115</b>	0.006	0.000	0.000	0.000	0.000	0.000
<b>117</b>	0.006	0.000	0.000	0.000	0.025	0.000

<b>FCB1</b>						
<b>Allele Size (bp)</b>	<u>Gulf of Mexico</u>		<u>Mediterranean Sea</u>		<u>North Sea</u>	
	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>
<b>119</b>	0.072	0.113	0.250	0.250	0.100	0.111
<b>121</b>	0.373	0.388	0.205	0.063	0.200	0.222
<b>123</b>	0.102	0.088	0.068	0.188	0.050	0.056
<b>125</b>	0.048	0.100	0.136	0.125	0.075	0.056
<b>129</b>	0.048	0.025	0.000	0.000	0.100	0.083
<b>131</b>	0.090	0.100	0.023	0.063	0.100	0.083
<b>133</b>	0.090	0.063	0.114	0.125	0.200	0.194
<b>135</b>	0.084	0.063	0.091	0.125	0.100	0.111
<b>137</b>	0.078	0.063	0.114	0.063	0.025	0.028

Table A.II.1

FCB1 (cont.)		Gulf of Mexico		Mediterranean Sea		North Sea	
Allele Size (bp)		All	Restricted	All	Restricted	All	Restricted
<u>139</u>		0.006	0.000	0.000	0.000	0.000	0.000
<u>141</u>		0.000	0.000	0.000	0.000	0.050	0.056
<u>143</u>		0.006	0.000	0.000	0.000	0.000	0.000

FCB14		Gulf of Mexico		Mediterranean Sea		North Sea	
Allele Size (bp)		All	Restricted	All	Restricted	All	Restricted
<u>282</u>		0.012	0.013	0.000	0.000	0.050	0.056
<u>286</u>		0.223	0.225	0.235	0.250	0.250	0.250
<u>288</u>		0.012	0.000	0.000	0.000	0.000	0.000
<u>292</u>		0.006	0.000	0.000	0.000	0.000	0.000
<u>294</u>		0.066	0.038	0.029	0.000	0.025	0.000
<u>298</u>		0.271	0.300	0.235	0.250	0.225	0.222
<u>300</u>		0.139	0.138	0.118	0.083	0.200	0.194
<u>302</u>		0.163	0.188	0.147	0.083	0.175	0.194
<u>304</u>		0.072	0.075	0.147	0.083	0.025	0.028
<u>308</u>		0.036	0.025	0.088	0.250	0.050	0.056

FCB17		Gulf of Mexico		Mediterranean Sea		North Sea	
Allele Size (bp)		All	Restricted	All	Restricted	All	Restricted
<u>140</u>		0.048	0.050	0.182	0.250	0.050	0.056
<u>146</u>		0.012	0.025	0.000	0.000	0.000	0.000
<u>156</u>		0.018	0.025	0.000	0.000	0.100	0.083
<u>158</u>		0.133	0.138	0.000	0.000	0.150	0.167
<u>160</u>		0.193	0.175	0.068	0.063	0.100	0.111
<u>162</u>		0.072	0.088	0.432	0.438	0.050	0.056
<u>164</u>		0.072	0.088	0.000	0.000	0.175	0.167
<u>166</u>		0.054	0.038	0.000	0.000	0.050	0.056
<u>168</u>		0.042	0.013	0.045	0.063	0.050	0.056
<u>170</u>		0.006	0.013	0.068	0.063	0.050	0.056
<u>172</u>		0.072	0.075	0.000	0.000	0.000	0.000
<u>174</u>		0.048	0.075	0.000	0.000	0.025	0.028
<u>176</u>		0.084	0.063	0.136	0.125	0.100	0.056
<u>178</u>		0.018	0.013	0.000	0.000	0.000	0.000
<u>180</u>		0.018	0.013	0.045	0.000	0.025	0.028
<u>181</u>		0.030	0.025	0.000	0.000	0.000	0.000
<u>183</u>		0.030	0.038	0.023	0.000	0.025	0.028
<u>185</u>		0.018	0.013	0.000	0.000	0.025	0.028
<u>187</u>		0.006	0.000	0.000	0.000	0.025	0.028
<u>189</u>		0.024	0.038	0.000	0.000	0.000	0.000

**Table A.II.1**

<b>GATA28</b>						
<b>Allele Size (bp)</b>	<u>Gulf of Mexico</u>		<u>Mediterranean Sea</u>		<u>North Sea</u>	
	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>
<b>120</b>	0.380	0.400	0.477	0.500	0.450	0.472
<b>128</b>	0.434	0.425	0.409	0.438	0.425	0.389
<b>132</b>	0.187	0.175	0.114	0.063	0.125	0.139

<b>GATA417</b>						
<b>Allele Size (bp)</b>	<u>Gulf of Mexico</u>		<u>Mediterranean Sea</u>		<u>North Sea</u>	
	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>	<i>All</i>	<i>Restricted</i>
<b>170</b>	0.120	0.088	0.045	0.063	0.100	0.111
<b>182</b>	0.651	0.663	0.795	0.813	0.550	0.500
<b>184</b>	0.012	0.000	0.000	0.000	0.025	0.028
<b>186</b>	0.217	0.250	0.159	0.125	0.325	0.361

